Note on Electronic File:

This document includes an electronic copy of the glyphosate-tolerant creeping bentgrass petition (USDA petition #03-104-01p), which was submitted to USDA/APHIS on April 14, 2003. The page numbers in this electronic version (with confidential business information deleted) differ from the submitted paper copy because of formatting. In this electronic copy, pages 2-434 contain the formal petition.

On pages 435-450 are copies of a letter (October 21, 2003) from USDA/APHIS/BRS to Monsanto and Scotts concerning the petition, and their letter of reply (November 10, 2003). The letters provide clarification and are a part of the Federal review process.

Following those letters, pages 451-474 contain additional information that was provided by Monsanto and Scotts on December 29, 2003, as an amendment* to the original petition. *This additional information has not yet been reviewed by the USDA/APHIS, as it was received after preparation of the preliminary risk assessment and the *Federal Register* notice which was published on January 5, 2004 (69 FR 315-317).

Petition for Determination of Nonregulated Status:

Roundup Ready® Creeping Bentgrass (*Agrostis stolonifera* L.) Event ASR368

The undersigned submits this petition under 7 CFR Part 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR part 340.

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Submitted April 11, 2003

Petition # 01-TR-054U

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CONTAINS NO CONFIDENTIAL BUSINESS INFORMATION

Additional Information Provided to USDA

- Summary of Alternative Pesticides for Removal of Roundup Ready Creeping Bentgrass in Undesired Areas on Golf Courses, Grass Seed Production, and other Crop and Non-crop Areas
- Pest Risk Assessment for Agrostis stolonifera L. Creeping Bentgrass, genetically modified to include glyphosate resistance by Dr. Alan Tasker, PPQ, APHIS, USDA
- Expert Letter from Dr. Zac Reicher, Associate Professor, Turfgrass Extension Specialist, Purdue University
- Expert Letter from Dr. Stephen Hart, Assistant Extension Specialist, Rutgers University
- Expert Letter from Dr. Suleiman Bughara, Assistant Professor, Turf Grass Geneticist and Breeder, Michigan State University
- Poster Abstract by Carson et al. entitled “Selective Control of Creeping Bentgrass in True Putt Creeping Bluegrass”, presented at the 2003 American Society of Agronomy Annual Meeting

Petition For Determination of Nonregulated Status for Roundup Ready® Creeping Bentgrass (*Agrostis stolonifera* L.) Event ASR368

Summary

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (7 CFR U.S.C. sections 7701-7772), to prevent the introduction and/or dissemination of plant pests into the United States or interstate introduction and/or dissemination. The APHIS regulations, at 7 C.F.R. § 340.6, provide that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company and The Scotts Company are submitting this request for a determination of nonregulated status to APHIS for Roundup Ready creeping bentgrass (Agrostis stolonifera L.) that is tolerant to glyphosate, the active ingredient in Roundup® industrial turf and ornamental (IT&O) herbicides. The glyphosate tolerance of Roundup Ready creeping bentgrass event ASR368 (event ASR368) is imparted by the insertion of a 5-enolpyruvylshikimate-3-phosphate synthase (epsps) gene from Agrobacterium sp. strain CP4 (cp4 epsps) into the creeping bentgrass genome. When creeping bentgrass plants containing the inserted gene are treated with glyphosate, the plants are unaffected since the continued action of the expressed tolerant CP4 EPSPS enzyme provides the plant's need for aromatic amino acids. The CP4 EPSPS protein as expressed in event ASR368 is identical to that expressed in Roundup Ready soybean (96-317-01p) and other Roundup Ready crops, all previously reviewed and granted nonregulated status by the USDA.

Creeping bentgrass with glyphosate tolerance will enable seed producers and golf course owners and superintendents to utilize Roundup IT&O herbicides such as Roundup PRO® herbicide for effective control of weeds occurring in the production of grass seed and to maintain superior quality turf on golf courses. Roundup PRO herbicide is highly effective against the majority of annual and perennial grasses and broadleaf weeds common to grass and turf production and has excellent environmental features, such as rapid soil binding as well as low toxicity to mammals, birds and fish. In addition, glyphosate is one of the few herbicidal active ingredients classified as "Category E" by the EPA (evidence of non-carcinogenicity for humans).

The use of Roundup Ready creeping bentgrass can benefit current agronomic practices in creeping bentgrass seed production and its use as a principal turf on golf courses by: (1) offering the seed producer and golf course superintendent a new, wide-spectrum weed control option that will increase the purity of creeping bentgrass seed and turf through improved broadleaf and grass weed control; (2) increasing flexibility to treat weeds on an

"as needed" basis; (3) allowing seed production on marginal land with severe grass weed infestations and (4) enhancing the uniformity, quality, aesthetics and playability of golf course turf.

The donor organism, *Agrobacterium* sp. strain CP4, was used to supply the cp4 epsps coding sequence for glyphosate tolerance. The CP4 EPSPS protein is well characterized and is homologous to plant and microbial EPSPSs, which are widely prevalent and have a long history of safe use. The transformation vector, PV-ASGT08L, containing the cp4 epsps gene and regulatory sequences, was introduced into the creeping bentgrass genome utilizing particle bombardment to produce event ASR368.

Molecular analysis of event ASR368 was performed to characterize the single stable site of insertion into the plant genome. Southern blot analyses confirmed that event ASR368 contains one copy of the transformation cassette inserted at a single locus in the plant genome. No additional elements from the DNA linear fragment, linked or unlinked to intact gene cassettes, were detected in the plant genome. Event ASR368 does not contain any detectable plasmid backbone sequence. These data support the conclusion that the insert in event ASR368 will only encode the single additional protein of interest, CP4 EPSPS.

Segregation analysis for nineteen creeping bentgrass populations derived from the reciprocal crosses made between event ASR368 F1 progeny and non-transgenic elite parental plants confirmed the heritability and stability of the cp4 epsps gene. Southern blot fingerprint analysis of DNA extracted from plants spanning three generations further confirmed the stability of the inserted gene in event ASR368. Plant production of CP4 EPSPS protein was determined in forage tissue collected from replicated field trials. Levels were found to be in the range of 64.1 to 77.1 µg/g of fresh weight for forage tissue. This low level of CP4 EPSPS protein expression in event ASR368 is sufficient to confer high levels of glyphosate tolerance.

Agronomic, morphological and pest susceptibility observations have been recorded in multiple field trials conducted in key production areas for creeping bentgrass in the U.S. These trials confirmed that event ASR368 is phenotypically equivalent to non-transgenic creeping bentgrass except for tolerance to glyphosate. As such, neither the *cp4 epsps* gene, including the regulatory sequences, nor the CP4 EPSPS protein, confers any plant pest characteristics.

There is no reason to believe that event ASR368 would have any significant adverse effect to organisms beneficial to plants or to non-target organisms, including threatened or endangered species. The safety of the EPSPS family of proteins, and specifically CP4 EPSPS as produced in a number of Roundup Ready crops, including corn, soybean, canola, cotton and sugar beet, has been demonstrated. The agronomic consequences of volunteer creeping bentgrass would be minimal because the plants are easily controlled by mechanical means or by one of a number of other currently registered herbicides.

Data and information in this request demonstrate that event ASR368 does not represent a unique plant pest risk. Therefore, Monsanto Company and The Scotts Company request a determination from APHIS that event ASR368 and any progenies derived from crosses between lines of this event with other bentgrass varieties no longer be considered regulated articles under regulations in 7 CFR part 340.

Roundup, Roundup Ready and Roundup PRO are registered trademarks of Monsanto Technology LLC.

Certification

The undersigned certify that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioners, which are unfavorable to the petition.

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Abbreviations, Definitions and Acronyms

~ .....Approximately
§.....Section
3' .....The distal, or growing end, of an mRNA transcript; the end nearest to or containing the polyA sites
5' .....The proximal, or start end, of an mRNA transcript; the end nearest to the promoter
A.....Adenine
A. stolonifera.....*Agrostis stolonifera*
AOSA.....Association of Official Seed Analysts
AOSCA.....Association of Official Seed Certification Agencies
bp.....Nucleotide base pairs
B99061R.....non-transgenic tissue culture line also referred to as B99061R/99028
C.....Cytosine
ca. .....Approximately
CaMV.....Cauliflower mosaic virus
CP4 EPSPS .....EPSPS protein from *Agrobacterium* sp. strain CP4
cp4 epsps.....Gene encoding the CP4 EPSPS protein (enzyme)
CTAB.....Cetyltrimethylammonium bromide
CTP .....Chloroplast Transit Peptide
*ctp*2.....DNA sequence coding for CTP variant 2
DNA.....Deoxyribonucleic acid
dATP.....Deoxyadenosine triphosphate
dCTP .....Deoxycytidine triphosphate
E9 3'.....3' polyadenylation region of the pea *rbc*S E9 gene
E. coli .....*Escherichia coli*
EDTA.....Ethylenediaminetetraacetic acid
ELISA .....Enzyme-linked immunosorbent assay
EPSP 5-Enolpyruvylshikimate-3-phosphate
EPSPS .....5-Enolpyruvylshikimate-3-phosphate synthase
G.....Guanine
HCl.....Hydrochloric acid
NPTII .....Neomycin phosphotransferase II
Kb.....Nucleotide kilobase pairs
kD.....kiloDalton
LB.....Left Border
M.....Molar
Ml.....milliliter
mM .....millimolar
mRNA.....Messenger RNA
MW .....Molecular weight

Abbreviations, Definitions and Acronyms[1] (continued)

NaCl .....Sodium chloride
NaOH .....Sodium hydroxide
Na_2HPO_4 .....Sodium phosphate dibasic
NOS.....Nopaline synthase
OECD.....Organization for Economic Co-operation and Development
ori .....Origin of replication
ori-V.....Bacterial origin of replication from RK2 plasmid
ori-322.....Bacterial origin of replication from *E.coli* plasmid pBR322
OSCS.....Oretgon State University Seed Certification Service
OSU.....Oregon State University
PCR .....Polymerase chain reaction
PEP.....phosphoenolpyruvate
PV-ASGT08L .....DNA plasmid vector used to transform event ASR368
RB .....Right Border
rpm .....Revolutions per minute
RR.....Genotype that is homozygous for glyphosate tolerance
Rr.....Genotype that is hemizygous for glyphosate tolerance
rr .....Genotype that is homozygous for glyphosate sensitivity
RT .....Room temperature
S3P Shikimate-3-phosphate
SDS .....Sodium dodecyl sulfate
sp. .....Species
spp .....Sub-species
SSC .....Saline-sodium citrate buffer. 20X SSC is 3 M sodium chloride, 0.3 M sodium citrate
T .....Thymine
T-DNA .....Transferred DNA
TE buffer.....Tris-EDTA buffer (10 mM Tris, pH 8.0, 1 mM EDTA)
TMB.....(3,3',5,5' Tetramethylbenzidene) peroxidase substrate
Tris .....Tris (hydroxymethyl)aminomethane
U.S.C. .....United States Code

[1]Standard abbreviations, e.g., units of measure, will be used according to the format described in 'Instructions to Authors' in the *Journal of Biological Chemistry.*

Request for Determination of Nonregulated Status
for Roundup Ready Creeping Bentgrass Event ASR368

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I. Rationale for Submission of Request for Determination of Nonregulatory Status

A. Basis for request for a determination of nonregulated status under 7 CFR Part 340.6.

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (7 U.S.C. sections 7701-7772), to prevent the introduction and dissemination of plant pests into the United States or interstate introduction or dissemination. The APHIS regulations, at 7 C.F.R. Part 340.6, provide that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

B. Roundup Ready® creeping bentgrass event ASR368

The Monsanto Company and The Scotts Company have developed Roundup Ready creeping bentgrass event ASR368 (event ASR368) that is tolerant to glyphosate, the active ingredient in Roundup® industrial, turf and ornamental herbicides (Roundup IT&O herbicides). The EPSPS enzyme from *Agrobacterium* sp. strain CP4 is functionally similar to plant EPSPS enzymes but has a greatly reduced affinity for glyphosate (Padgette *et al*., 1996). In non-transgenic plants, glyphosate binds to the plant EPSPS enzyme and blocks the biosynthesis of aromatic amino acids thereby preventing plant production of these essential compounds (Steinrucken and Amrhein, 1980; Padgette *et al*., 1996). In Roundup Ready creeping bentgrass, metabolic requirements for the plant's growth and development are met by the continued action of the glyphosate tolerant CP4 EPSPS enzyme in the presence of glyphosate.

Creeping bentgrass with glyphosate tolerance will enable the use of Roundup IT&O herbicides, such as Roundup PRO herbicide, for effective control of weeds occurring in the production of grass seed and to maintain superior quality turf on golf courses. Roundup PRO herbicide is highly effective against the majority of annual and perennial weeds common to grass seed and turf production. Roundup herbicide also has excellent environmental features, such as rapid soil binding (making it resistant to leaching), as well as low toxicity to mammals, birds and fish. In addition, glyphosate is one of the few herbicidal active ingredients classified as "Category E" by the EPA (evidence of non-carcinogenicity for humans) (57 FR 8739).

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C. Benefits of Roundup Ready creeping bentgrass

C.1. Benefits to the golf course industry

An analysis of the potential benefits of Roundup Ready creeping bentgrass to the golf course industry is provided in Appendix I. A summary of these benefits follows.

Creeping bentgrass is characterized as a low-growing, fine-textured, soft, very dense, carpet-like turfgrass sward that tolerates low mowing. When used as a close-mowed turf, frequent watering, optimum fertilization, disease management and soil management practices are needed to prevent competition from other grass or broad-leafed weed species. Even under optimal nutrition and watering regimes, creeping bentgrass is susceptible to a wide range of diseases, including pink snow mold, brown patch and dollar spot. Therefore, because of the intense level of management required, creeping bentgrass is rarely employed for residential use but rather for golf course putting greens, tees and fairways, lawn bowling greens, grass tennis courts and other specialized applications. Monsanto and The Scotts Company intend to market event ASR368 exclusively to the professional market for seed and sod production for use solely on golf courses. This product will not be sold for residential, industrial or other recreational applications.

For over 100 years, creeping bentgrass has been planted on golf courses in the cool season turfgrass growing areas of North America. It is estimated that 11,600 U.S. golf courses now manage ca. 24,400 acres of creeping bentgrass putting greens, or an average of 2.1 acres per course (Appendix I). In addition, about 16,140 golf courses are in operation, which suggests that creeping bentgrass is grown on approximately 70% of U.S. golf putting greens (Appendix I). It is also estimated that fairways accounted for 52,900 acres of creeping bentgrass on over 2,300 golf courses for an average of 23 acres per course, which represents about 15% of U.S. fairways (Appendix I).

Adoption of Roundup Ready creeping bentgrass by golf courses can both simplify and improve the efficacy of weed control. A wide array of annual and perennial grass, broadleaf and sedge species can invade golf turf. These pests are currently controlled with variable success using a variety of herbicides and plant growth regulators (PGRs) that are applied throughout the growing season. These products number in the dozens and range from phenoxy acid and arsenical compounds to more recently introduced products such as ethofumesate, quinclorac and plant growth regulators such as paclobutrazol for annual bluegrass suppression.

C.1.a. Environmental and health benefits

An over-the-top treatment with Roundup PRO herbicide will significantly reduce the need for many of these other herbicides. Exceptional cases may include the control of especially difficult weeds species, managing the unlikely development of a weed population resistant to glyphosate or specific situations where the use of Roundup PRO herbicide is inappropriate. In addition, certain fungicide and insecticide uses to manage

pests of annual bluegrass (*Poa annua)* may be reduced when annual bluegrass is removed from mixed-species turfgrass stands (Appendix I).

Displacement of other pesticides with Roundup PRO herbicide will potentially reduce risks to human health and the environment. The toxicological, carcinogenicity, leaching, and runoff characteristics of glyphosate formulated for golf course use (Roundup PRO herbicide), translate into a reduced potential risk for this technology versus other products commonly used on creeping bentgrass (Appendix I).

Roundup PRO herbicide has the fewest label warnings and least restrictive use requirements compared to other herbicides registered for use on golf courses, which reflects its overall lower risk of causing adverse effects to applicators and the environment as shown in Appendix I. Furthermore, glyphosate, the active ingredient in Roundup herbicide is classified as a Category E carcinogen, which is the lowest carcinogenicity risk assigned by the EPA and indicates that it is not a carcinogen. The vast majority of commonly used golf course pesticides have a carcinogenicity classification indicative of higher risk than glyphosate (Appendix I).

Glyphosate does not exceed the regulatory Level of Concern in worst-case risk analysis scenarios for fish, aquatic invertebrates, and aquatic plants. Hence, the Roundup Pro label, unlike most other important golf course herbicides, does not require warnings of toxicity to fish and/or aquatic invertebrates and/or non-target plants.

C.1.b. Annual bluegrass *(Poa annua)* management benefits

Particularly challenging grass weed problems in creeping bentgrass include annual bluegrass and rough stalk bluegrass (*Poa trivialis*) because of their similar ecological adaptations and fairway bermudagrass (*Cynodon dactylon*) encroachment onto creeping bentgrass greens during heat stress periods. Overall, annual bluegrass is the most pervasive and troublesome weed in highly managed creeping bentgrass because it thrives and disperses viable seed under the same mowing, irrigation, and fertilization regimes as creeping bentgrass grown for fairway and putting green uses. In spite of its susceptibility to disease and environmental stress, annual bluegrass may be maintained as a playing surface in a very limited number of golf course environments because of its density, vigor and tolerance to shade and close mowing.

However, in most environments, annual bluegrass suffers from a variety of cold hardiness, heat tolerance and pest susceptibility problems that limit its utility as a perennial turfgrass. Most importantly, annual bluegrass frequently fails under the heat and drought stress conditions of midsummer because it is best adapted to cool, moist conditions. The result of its aggressive cool-season colonization and marginal warm-season survivability is that golf course superintendents invest a great deal of labor, chemistry, water and time into managing annual bluegrass in mixed stands with creeping bentgrass either in a futile attempt to eliminate it or to encourage its survival in situations where control strategies have failed.

Just the potential elimination of annual bluegrass with Roundup PRO herbicide from Roundup Ready creeping bentgrass fairways offers numerous associated agronomic and environmental advantages (Appendix I). According to University estimates, if all creeping bentgrass greens, tees and fairways were converted to Roundup Ready bentgrass the following may result:

- potential reduction of 400,000 pounds of pesticidal active ingredient or the equivalent of 1.1 million pounds of a 35% active ingredient product;
- overall reduction in fungicide, plant growth regulator and herbicide active ingredient of ca. 20% for greens, tees and fairways combined, or 19.4 pounds of active ingredient per acre;
- potential reduction in fungicides of 11.6 pounds of active ingredient per acre for greens, tees and fairways combined;
- potential reduction of herbicides and plant growth regulators such as methyl bromide of 7.1 and 0.7 pounds of active ingredient per acre, respectively, for greens, tees and fairways combined; and
- improved creeping bentgrass performance as a result of single-species management focus.

The varying degree to which annual bluegrass management impacts individual golf course operations makes assessing the overall benefits of Roundup Ready creeping bentgrass difficult. In certain cases, drastically different approaches to managing pure creeping bentgrass may be adopted, such as the complete elimination of fumigants at establishment, such as methyl bromide, and shifts in cultural programs. In other cases, the availability of the technology may prompt conversions from other species with their own management challenges.

C.1.c. Additional turfgrass management benefits

General changes to weed control and turfgrass management from the adoption of Roundup Ready creeping bentgrass can produce these additional benefits:

- reduced creeping bentgrass injury from the use of marginally selective herbicides;
- labor reductions from reduced pesticide applications and annual bluegrass cultural management; and
- increased golfer (customer) satisfaction from improved playability and performance of fairway surfaces.

D. Benefits to creeping bentgrass seed production

To meet the demand for creeping bentgrass seed used by U.S. golf courses, as many as 7,000 acres are farmed for seed production annually. To satisfy the anticipated demand by seed farms and golf courses for Roundup Ready creeping bentgrass seed we believe that considerably less acreage will be needed, which can be grown by a small number of

growers. Although commercialization of Roundup Ready creeping bentgrass is likely to have considerable value to those seed producers growing this crop, the greatest benefit of this technology will result from its use on golf courses. Nonetheless, it is expected that benefits accruing to Roundup Ready creeping bentgrass seed producers will include:

- Increased purity of creeping bentgrass seed through better weed control of competing grasses;
- The option to use an environmentally preferred herbicide that will reduce worker exposure to herbicides with less favorable toxicological characteristics;
- An overall reduction in herbicide usage through an increased flexibility to treat weeds on an as needed basis; and
- An ability to produce seed on land considered marginal due to severe infestations of grassy weeds such as annual bluegrass, roughstalk bluegrass, quackgrass (*Agropyron repens*) or bromegrass (*Bromus* species).

E. Regulatory clearance of Roundup Ready creeping bentgrass

Before commercializing Roundup Ready creeping bentgrass event ASR368 in the U.S., Monsanto and The Scotts Company will obtain the following:

1. A determination of nonregulated status from USDA APHIS for Roundup Ready creeping bentgrass event ASR368 and all progenies from crosses between lines containing this event and other creeping bentgrass varieties. As a result, this creeping bentgrass line and progenies would no longer be regulated articles according to 7 CFR Part 340.6.

2. Creeping bentgrass is not consumed by humans and only the straw and chaff have limited use as animal feed. Therefore, event ASR368 is within the scope of the FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, published in the Federal Register on May 29, 1992. As a result of consultations on Roundup Ready creeping bentgrass with the FDA since May 2001, Monsanto and The Scotts Company provided a summary of the animal feed safety and nutritional assessment of event ASR368 to the Agency on September 13, 2002 to permit the feed use of Roundup Ready creeping bentgrass straw and chaff.

3. In January 2002, a proposed supplemental label for Roundup PRO Herbicide (EPA Reg. No. 524-475) for uses in seed production of Roundup Ready creeping bentgrass was submitted to EPA for review and approval. A separate supplemental label for Roundup PRO Herbicide for general weed control in Roundup Ready creeping bentgrass turf, planted to golf course tees, greens and fairways, was submitted to EPA for approval in May 2002.

F. References

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II. The Bentgrass Family

The following section provides an in-depth review of the scientific literature regarding the origin, use, biology, taxonomy, genetics, related species and potential weediness of creeping bentgrass. Although a wind-pollinated and an obligate outcrossing species, this review provides substantial evidence to support the following statements regarding *A. stolonifera*:

- seed has been successfully produced in the U.S. for more than 100 years and for the past 75 years in essentially a single defined geographic area without becoming either an uncontrolled weed or important contaminant of mpre than 80 other turfgrass species;
- the formation of hybrids with related species is rare and declines precipitously with increasing distance from a source plant;
- hybrids are typically intermediate of their parents and predominantly sterile; and
- the species neither exhibits characteristics of a weed nor has a history of weediness.

Furthermore, when considered in conjunction with the data and information provided in Section VI of this document, it is apparent that event ASR368, is no more likely to become a weed than conventional creeping bentgrass.

A. Origin and cultural history of *A. stolonifera*

A.1. Geographic origin

Complete agreement in the literature as to the geographical origin of *A. stolonifera* does not exist. However, the species is thought to have originated in cooler climes of both Eurasia and North America (Hitchcock, 1950; Hubbard, 1984) but is now naturalized in similar environments in other regions of the world (Hubbard, 1984; Hitchcock, 1950). Native and naturalized habitats of *A. stolonifera* tend to be moist and humid and include coastal areas (Hitchcock, 1950; Hubbard, 1984), highland meadows (Hubbard, 1984), flood plains (Bradshaw, 1958a) and early succession forested areas (Collet *et al*., 1996). It apparently has been a common component of Eurasian pasturelands for centuries (Bradshaw, 1958a; King, 1962; Duich, 1985).

A.1.a. Sporting uses of *A. stolonifera*

A. stolonifera has been planted as a playing surface on golf course putting greens, tees and fairways in cool season turfgrass growing areas of North America for over 100 years (Duich, 1985; Hurley and Murphy, 1996). There is practically no current home lawn or institutional market for *A. stolonifera* because of the high level of management required to maintain its aesthetic character (Hanson *et al.*, 1969; Turgeon, 2002).

The tolerance of *A. stolonifera* to extremely close mowing and its stoloniferous, spreading habit make it especially well adapted to the frequent, close mowing and recuperative requirements of golf course putting greens and modern fairways (Reese, 2000). *A. stolonifera* excels and is most competitive at mowing heights between 0.125 and 0.75 inch that are common to golf course greens and fairways, and is not reported to produce mature flowers, shed pollen nor produce viable seed that are disruptive to play at these mowing heights (Lush, 1988a; Quemada, 1999). Its vegetative growth response to consistent water, nitrogen-based fertilizers and sunlight allows it to recover from the damage due to traffic and golf club action (Beard, 2002). Surrounding areas (golf course roughs) are typically planted to turfgrasses of different genera with less intensive management inputs (Beard, 2002).

A.1.b. Cultural history

Although potentially native to this continent (Hitchcock, 1950), a North American origin of the *A. stolonifera* varieties used for golf course green turf has been questioned (Duich, 1985; Meyer and Funk, 1989; Hurley and Murphy, 1996). The earliest *A. stolonifera* golf putting greens were established in the U.S. by greens keepers who imported "South German" bentgrass seed in the late 19th century from Europe (Duich, 1985).

South German bentgrass was an inconsistent mixture of *Agrostis* species, primarily colonial bentgrass (*Agrostis tenuis* Sibth now accepted as *A. capillaris* L.) variably mixed with velvet bentgrass (*Agrostis canina* L.), redtop (*Agrostis alba* L., now accepted as *A. gigantea* Roth.) and typically only a trace of *A. stolonifera*. That trace was sufficient, however, to produce greens of predominantly *A. stolonifera* or mixtures with *A. canina* over a period of 20 or 30 years (Huff and Landschoot, 2000). The selection and use of vegetatively propagated varieties of *A. stolonifera* from these mature greens began as early as 1917 (Holt and Payne, 1951) and was prompted in part as a means to mitigate the incidence of diseases common to other vegetative varieties (Hurley and Murphy, 1996). Popular vegetative varieties included 'Congressional', 'Toronto', 'Washington' and 'Cohansey', names that reflect the geographic or golf course source of the original selection (Meyer and Funk, 1989).

Interestingly, the variable mixture of South German bentgrass seed was harvested from naturalized *Agrostis* pastures in Europe where some frequency of *Agrostis* hybridization should have been expected. However, records or reports of *Agrostis* hybrid survival at the expense of typical *Agrostis* species started from seed on golf courses in the U.S. could not be found.

The breeding of and conversion to seeded *A. stolonifera* varieties began by the mid-1900s (Hurley and Murphy, 1996). The first commercially available Certified seeded variety, 'Seaside', became available in the 1920s. Hitchcock (1950) refers to Seaside bentgrass as a seeded variety of native origin, while Hurley and Murphy (1996) refer to it as a naturalized species, developed with no formal breeding. Regardless of its origin, Seaside and other early seeded *A. stolonifera* varieties were not improved through plant breeding

but harvested from nearly pure, naturally occurring stands (North and Odland, 1935; Schoth, 1930).

An entire edition of *The Bulletin*, a publication of the United States Golf Association Green Section, was devoted to early bentgrass seed production in North America. Articles recount some of the pioneers and techniques used by the bentgrass seed industry in the Pacific Northwest, Rhode Island and Canada (USGA, 1930). Odland (1930) reports that bentgrass is mentioned in U.S. records from at least 1747, although no reference is cited. In 1924, Lyman Carrier, USDA, traveled to the Coos Bay, Oregon region with H.A. Schoth, USDA, to look for creeping bentgrass stands identified by Schoth and Roland McKee of the Bureau of Plant Industry on a previous visit in 1923 (Schoth, 1930). They found nearly pure stands in dairy pastures along coastal streams. Don Brewer, former Oregon Seed Certification Director, recounted the early years of creeping bentgrass seed production in Oregon (Brewer, 1992; Oregon State University, personal communication, 2002). Brewer wrote that Carrier resigned from the USDA, then immediately moved to Oregon and formed a company called 'Cocoos' that would purchase bentgrass hay from the growers of the pure creeping bentgrass stands and then thresh, condition and market the seed as Cocoos bentgrass. Carrier was competing with imported German bentgrass that was being sold for $5.00 per pound.

Apparently, Professor George Hyslop at Oregon State University learned of Carrier's operation and felt growers were losing a valuable opportunity. Hyslop then apparently encouraged growers to harvest and process the seed themselves and offered OSU to "certify" their seed as Seaside variety creeping bentgrass (Brewer, 1992; OSU personal communication, 2002). Hyslop (1930) also recounts the difficulty in distinguishing redtop and creeping bentgrass seed at the time. The creeping bentgrass, Seaside, was first harvested in 1926 and the first Certified Seaside creeping bentgrass was harvested in 1927 (Hyslop, 1930).

The first seeded variety improved through breeding and selection was Penncross, introduced by the Pennsylvania State University in 1954 (Duich, 1985). The breeding of seeded varieties has progressed considerably, with more than 25 new varieties released in the last 20 years (Cattani *et al*., 1996; Hurley and Murphy, 1996). Some of the selection and breeding of current cultivars was the result of crossbreeding of early named vegetative selections along with other high quality clones from turf areas or conventional cultivars such as Penncross and selecting and reselecting among the progeny resulting from these crosses.

A.2. Commercial production of *A. stolonifera*

A. stolonifera is grown commercially either for athletic playing surfaces – primarily golf – or for seed production to supply the golf industry. A recent market research survey (Doane Agricultural Services, 1999) estimated that 11,600 U.S. golf courses manage a collective 24,400 acres of *A. stolonifera* for putting greens and that 2,300 golf courses manage 52,900 acres for fairways. Between five and seven thousand acres of *A.*

stolonifera seed production eligible for seed certification standards have been grown annually in Oregon since 1996 (Oregon State University, 2001a). Seed production is centered in the Willamette Valley (Oregon Department of Agriculture, 2001a), where climate and irrigation availability are favorable to *A. stolonifera* survival and optimum seed yields.

A. stolonifera is established from either seed or stolons according to the variety and use. Golf courses are most commonly seeded, but can also be established with sod from a nursery area or sod farm. Sod from a commercial supplier or an onsite nursery may be used for limited installations or for larger emergency repairs. Seed production fields are most commonly established with seed but Penncross fields must be established with alternating rows of three certified vegetatively propagated parents which inter-pollinate in order to produce the commercially available seed (Oregon State University, 2001a; Anonymous, 1958).

A. stolonifera occurs naturally in coastal meadow areas of New England and the Pacific Northwest and as a pasture component in moist, nutrient-rich settings of North America and Eurasia (Hitchcock, 1950; Bradshaw, 1958a). It is almost exclusively a volunteer colonizer in these instances and does not perform well as forage compared to sown grasses such as perennial ryegrass and tall or fine fescue (Frame, 1990).

A.2.a. Establishment

Establishment strategies for *A. stolonifera* on golf courses focus on high populations and the most rapid germination and grow-in possible, so that play may commence at the earliest possible date. *A. stolonifera* seeds are among the smallest grass seeds (6-7 million/pound) (Musser and Perkins, 1969) and therefore limited in carbohydrate reserves. Successful establishment of commercial *A. stolonifera* by seed requires a highly prepared seedbed and substantial inputs of water and nutrients to encourage germination and rooting (Burton, 1992).

Fall seeding or stolon sprigging is preferred over spring for seed production because floral induction of buds is required to promote reproductive floral initiation the following spring (Turgeon, 2002). The economic outcome of this physiological process is that in some geographic areas fall seedings produce a crop in approximately twelve months instead of eighteen for spring seedings. Fall establishment also decreases annual weed pressure and provides a longer development period before the following summer golf season (White, 2000; Schumann *et al*., 1998).

A. stolonifera prefers a slightly acid soil pH between 5.5 and 6.5. Soil acidity should be adjusted prior to planting according to a soil test (Beard, 2002). Likewise, soil nutrient levels are adjusted both prior to planting based on a soil test and then frequently after establishment based on either a soil or foliar test (Beard, 2002). Mature fairway plantings require between 80 and 160 pounds of nitrogen per acre per season and greens are generally fertilized at 1.5 times the fairway recommendation (Beard, 2002). Newly

established stands are fertilized more intensively and frequently than mature stands to hasten root growth, plant development and to establish a tight closed plant stand (Beard, 2002).

Seeding rate recommendations for new turfgrass establishment of *A. stolonifera* range from as low as 20 to 40 seeds per square inch or 0.5 to 1.0 pound per 1000 square feet (Madison, 1966) to as high 3.0 pounds per 1000 square feet (White, 2000; Beard, 2002) for some putting greens. The lower range of rates is most commonly recommended and will provide an adequate stand but some practitioners believe the higher rates will provide a denser stand in less time. Evidence suggests, however, that increased spatial competition in these plantings can lead to higher disease rates (Rossi *et al.*, 1999) and increased consumption of water and nutrients and that the presumed temporal benefit is seldom achieved (White, 2000). Lush (1990) proposed the use of the power rule to ascertain the potential wear tolerance of turfgrasses. Cattani (2000), using this formula, showed that wear resistance potential equilibrated by 12 weeks after seeding different rates of creeping bentgrass seed, even though there were distinct visual differences in the turf.

A.2.b. Plant breeding and commercial varieties

Modern *A. stolonifera* varieties are predominantly of synthetic origin, i.e., a number of selected parents are allowed to intercross in isolation (Hurley and Murphy, 1996). Seed is harvested from the clones and increased through to Certified status if desired as described below. The number of parents can vary from 4 to as many as 203 (Anonymous, 1985; Hurley *et al*., 1994). Other cultivars have been developed through somewhat different strategies. For example, Penncross is sold as the S_1 progeny of a field cross between three parents (Anonymous, 1958), while Putter utilizes a single pollen parent (BPA-163) in alternating rows with 120 different improved lines to produce a highly heterogeneous Breeder seed (Brauen *et al*., 1993). Although the cross-pollination strategies for seeded varieties may vary, open pollination is a requirement in the production process (Bradshaw, 1958a).

A.2.b.1. Genetic purity

In addition to *A. stolonifera*, large acreages of other grasses such as perennial ryegrass (*Lolium perenne* L.), annual ryegrass (*Lolium multiflorum* Lam.), tall fescue (*Festuca arundinaceae* L.) and fine fescue (*Festuca sp.,* various) are grown in the Willamette Valley, making grass seed one of Oregon's largest agricultural industries (US Department of Agriculture, 1999). The production of Kentucky bluegrass (*Poa pratensis* L.) is not as significant as these other species in the Willamette Valley but is common to the Columbia River Valley, the Columbia Basin in Washington State and through much of northern Idaho to the Rathdrum Prairie.

This concentration of grass species and cultivars in a relatively constrained geography means that cultivar identity and seed purity require a coordinated effort to achieve. In

addition, the identity preservation and quality control of these species are a high priority for seed producers and for the industry's quality image. Seed certification is performed according to the purity standards of the Oregon State University Seed Certification Service (OSCS). This is the current mechanism used by grass seed producers to ensure product identity and to meet industry-established quality standards. Pedigreed seed must conform to maximum acceptable levels of foreign matter, crop and weed contaminants and genetic off-types in the commercial product. This process has successfully provided high quality grass seed to international markets for decades.

A.2.b.2. Pedigreed seed and plant material programs

The genetic purity and identity of seed and propagating plant material is essential to the quality of planting stock for growers and ultimately of agricultural products sold to consumers. The standards and concept of isolating fields for maintenance of genetic purity have been in existence since the 1940 (Hackleman and Scott, 1990). Seed-producing states in the U.S. and developed countries have established lead agencies for the development and administration of minimum genetic purity and commercial quality standards. U.S. agencies operate their programs under the international standardization umbrella of the Association of Official Seed Certifying Agencies (AOSCA), founded in 1919 as the International Crop Improvement Association. Current member agencies of AOSCA include 42 U.S. states, two Canadian provinces, New Zealand, Australia and Argentina (AOSCA, 2001).

Since most U.S. grass seed is grown in the Willamette and Columbia River valleys, the Oregon State Extension Service, through the activities of the OSCS, plays a lead role in establishing and administering purity and quality standards for commercial breeding stocks and planting seed. The expressed purpose of OSCS is "to provide … for the maintenance and increase of quality seed and propagating material of varieties grown and distributed in such a manner as to insure varietal purity through the appropriate application of (its) rules." These rules are provided most recently in the OSCS's handbook (Oregon State University 2001a).

The following summary of pedigree seed certification is adapted from the Handbook:

"Oregon certifies only those varieties/cultivars for which identification, purity, and quality standards have been previously established by the OSCS or after review and acceptance of another state's approved standards. An application to approve a new cultivar must provide sufficient information to establish its genetic origin and breeding procedure, uniqueness and distinguishing characteristics, production performance, regional adaptation and purpose, and stock maintenance procedures.

Certified pedigree seed may be of four classes. "Breeder seed" is the result of the initial parental crosses and is the original source of all other certified seed classes. "Foundation seed" is produced from fields planted with Breeder seed and "Registered seed" is produced from Foundation seed plantings. "Certified seed" is an end use

product that is produced from either Registered or Foundation seed plantings. In cases where market demand is limited, a variety may not have a Registered class. Seed that is substandard to any class is either rejected or may be labeled as Substandard for commercial sale.

Certification of any seed class relies on seedling, seed crop, and post-harvest notifications to and inspections by OSCS for adherence to genetic, land use, isolation, field management, and post-harvest handling standards. Seedling inspection includes appropriate field location and proper identification, genetic eligibility of the stock seed (or stolon), adherence to land preparation requirements, proper isolation from contamination sources, absence of prohibited weeds, and crop management practices. Seed crop inspection occurs prior to harvest and includes crop eligibility, e.g., a prior seedling inspection or equivalent; proper isolation from contamination sources, absence of prohibited weeds, and proper field management. Post-harvest notifications and inspections focus on identity preservation in transport, storage and packaging."

Specific OSCS certification standards for *A. stolonifera* include the following:

- identification of eligible varieties;
- five year rotation out of *Agrostis spp.* previous to planting unless the previous crop was the same, Certified cultivar;
- seedling and seed crop inspections;
- off-type variety limits and specific isolation distances for each certification class
- minimum standards for pure seed, weed seed and foreign matter content for each certification class; and
- minimum 85% germination rate.

The Certified seed designation is an assurance that all reasonable steps are taken between the initial parental cross and the delivery of commercial seed to ensure the genetic identity and purity of the grass seed and the practical elimination of any other physical and biological contaminants. All seed classes subsequent to the Breeder class are essentially controlled bulking steps for distribution to the grower community and for increasing supplies to final market volumes. The presence of a Certified seed "Blue Tag" assures the buyer that contents of the seed bag are as described on the tag relative to cultivar identity, presence of off-types and/or contaminants and minimum germination thresholds.

The advantage of managing *A. stolonifera* in a certification program goes beyond the purity of Roundup Ready *A. stolonifera*. The purity of all *A. stolonifera* cultivars is enhanced by the resulting prevention of gene flow between production areas and by reduced adventitious movement of both transgenic and conventional genes into the surrounding environment.

A.2.b.3. Commercial varieties

The Oregon State Seed Certification Service Handbook (Oregon State University, 2001a) recognizes 37 *A. stolonifera* cultivars eligible for certification as Breeder, Foundation or Registered seed:

Breeder, Foundation or Registered: Backspin, Brighton, Cato, Century, Cobra, Crenshaw, Imperial, Lofts L-93, Lopez, Mariner, National, Regent, ProCup, Seaside, Southshore, SR 1020, SR 1119, Trueline, Viper

Breeder or Foundation: 18th Green, Carmen, Penncross (stolons), Pennlinks, Prominent, Providence, Putter, Emerald

Breeder: Grand Prix, Penn A-1, Penn A-2, Penn A-4, Penn G-1, Penn G-2, Penn G-6, Penneagle, Princeville, Seaside II

Vegetative: Pennlu

Recent trade journal reviews identify a limited number of these cultivars as most popular with the golf industry (Hurley and Murphy, 1996; Reese, 2000). Cultivars vary in their successful adaptation to various environments and management regimes and may differ in their adaptations for fairway and cool and high temperature putting greens (Hurley and Murphy, 1996). These cultivars are listed in Table II.1, excerpted from Hurley and Murphy, 1996.

Table II.1. Popular bentgrass cultivars planted on U.S. golf courses

Variety / Cultivar	Year Released	Source
Seaside	1923	Oregon State University
Penncross	1955	Pennsylvania State University
Penneagle	1979	Pennsylvania State University
Pennlinks	1986	Pennsylvania State University
Cobra	1987	Rutgers University and International Seeds
SR 1020	1987	University of Arizona and Seed Research
Providence	1988	University of Rhode Island and Seed Research
Putter	1989	Washington State University
Southshore	1991	Rutgers University and Lofts Seeds
Cato	1993	Texas A&M University
Crenshaw	1993	Texas A&M University
Penn A-1	1995	Pennsylvania State University
Penn A-2	1995	Pennsylvania State University
Penn A-4	1995	Pennsylvania State University
Penn G-4	1995	Pennsylvania State University
Penn G-6	1995	Pennsylvania State University
L-93	1995	Rutgers University and Lofts Seed
Backspin	1999	Texas A&M University

A.2.c. *A. stolonifera* seed production

A. stolonifera seed has been produced in the Willamette Valley of Oregon since the 1920s (Schoth, 1930). Approximately 6,000 acres are typically in production in this area (Oregon State University, 2001a). *A. stolonifera* produces seed over a number of years in production fields and in nature. Production fields are most commonly seeded in the fall, harvested from late July to early August (Cattani *et al*., 1997) and maintained for 3 to 5 years before conversion to another crop.

Greatest seed yields are generally found in the year after establishment (North and Odland, 1935) and are linked to the amount of reproductive tillering. A tiller is defined as an independent plant crown and its associated leaves, arises from axillary buds (meristems) present at leaf axils and may have a vegetative or reproductive function according to a number of environmental influences (Turgeon, 2002). A reproductive tiller that successfully reaches sexual maturity will produce an inflorescence or flower, and die off after seed set. *Agrostis stolonifera* is open pollinated, highly self-incompatible and therefore, must cross with another individual to effectively produce viable seed.

Seed production and reproductive tillering in *A. stolonifera* vary according to variety and management practices in seed production fields (North and Odland, 1935; Smith and Cattani, 1993; Cattani *et al*., 1997). The importance and contribution of common agronomic inputs such as pH adjustment, fertilization and water management are fairly

obvious for any crop yield. However, the most significant yield factor after the first production year is post harvest management. These management practices encourage the stimulation of new reproductive tillers for the following year's crop as the harvested tillers are no longer productive. Seed growers promote new tillering by straw removal, and aggressively scalping the plants to remove all top growth.

The optimum timing and method of renovation for reproductive tiller stimulation has not been fully developed. Cattani *et al*. (1997) and Gossen *et al*. (1997) reported poor seed yields with late August to early September renovations in western Canada. Cattani *et al*. (1997) also reported a significant increase in seed yield with early August field renovations. *Agrostis stolonifera* has shown good August and September regrowth in a continuously harvested forage production trial (Haggar, 1976), suggesting that increasing tiller density may be a factor. This time frame corresponds to the increase in tiller density in golf putting green turf reported by Cattani *et al*. (1991). The pattern of these studies suggests that early renovation as close as possible to harvest may provide the greatest potential yield benefit.

Commercial yields of *A. stolonifera* seed vary with stand age, renovation success and common management inputs such as water, fertilization, pest management and pH adjustment. Average yields in Oregon across all *Agrostis* species are between 550 and 600 lb/a (Oregon Department of Agriculture, 2001). *A. stolonifera* yields were not provided in these reports and most *Agrostis* seed in Oregon is grown under irrigation. Irrigated trials in Canada (Gossen *et al*., 1997) had *A. stolonifera* yields of 280 lb/a and dryland production has ranged between 100 and 400 lb/a (Cattani *et al.*, 1997; Smith and Cattani, 1993). To achieve optimal yields due to the late maturity date, supplemental irrigation is often needed as in the Willamette Valley (Meyer and Funk, 1989).

B. Biological characteristics of *A. stolonifera*

B.1. Taxonomy

A. stolonifera is a flat or involute-leaved, stoloniferous, perennial species with stolons up to 200 cm in length and smooth stems from 5 – 100 cm. The panicle inflorescence is characteristic of genus *Agrostis*. The inflorescence ranges from 1 to 30 cm in length with branches spreading only at anthesis. They often bear green to purplish spikelets of 2 to 3 mm in length and consist of a single perfect floret (Pohl, 1953; Tutin, 1980), which opens in the morning (Cattani pers. comm., 2002; Davies, 1953). *A. stolonifera* is wind-pollinated and an essentially obligate outcrossing species (Bradshaw, 1958a).

Agrostis stolonifera has been ascribed to the family *Poaceae;* subfamily, *Pooideae;* tribe *Agrostideae* (Hitchcock, 1971). A recently accepted classification is as follows:

Family: Poaceae
Subfamily: Pooideae
Tribe: Poeae
Subtribe: Agrostidinae
Genus: Agrostis (Soreng *et al*., 2001)

Creeping bentgrass used for turf is currently accepted as *A. stolonifera* L. (Soreng *et al.,* 2001). The species has also been referred to as *A. palustris* (Huds.) (Beard, 1973; Christians, 1998; Meyer and Funk, 1989) or *A. stolonifera* var. *stolonifera* and A. *stolonifera* var. *palustris* (Philipson, 1937). Breeders and taxonomists continue using both *A. stolonifera* L. and *A. palustris* Huds. synonymously or in combination as the species name for creeping bentgrass. We use *A. stolonifera* L. in this paper because it is the oldest and currently accepted usage.

The common name, "creeping bentgrass", has been erratically and erroneously applied to several *Agrostis* species including *A. alba* or more commonly "redtop" (Beal, 1896; Budd and Best, 1964). Gould and Shaw (1968) misapplied *A. stolonifera* to redtop (*A. alba* syn. *A. gigantea*). Common name usages have also contributed to many of the taxonomic difficulties associated with *A. stolonifera* L. (Bradshaw, 1958b; Cattani, pers. comm., 2002).

The misapplication of both common and scientific names to the various *Agrostis spp.* is a common problem with this genus and difficult to discern when comparing different taxonomic references (Stuckey and Banfield, 1946). This is due to frequent taxonomic classification based on adaptation and phenotypic characters, which reflect observed differences by particular taxonomists (Philipson, 1937). For example, Hitchcock (1950) describes *A. palustris* as similar to *A. stolonifera* except in a few phenotypic characters including length of stolons and its propagation for turf use. However, stolon length appears to exhibit considerable variability. Cattani *et al*. (1996) reported significant internode length differences between commercial *A. stolonifera* varieties. Cattani (1999) also found differences in stolon and internode length between two *A. stolonifera* varieties that were reportedly closely related. In addition, Burg *et al*., (1979) reported the use of lemma and palea characteristics in species determination of *Agrostis spp.* with similar seed size but Davies (1953) uses palea length to divide the commonly occurring U.K. *Agrostis* sp. into two types.

Varied attempts to definitively characterize *Agrostis spp.* or any other species suggest that the use of phenotypic differences, while convenient, is not always clear and should not be relied upon as the sole method to distinguish between closely related species (Philipson, (1937; Barkworth and Dewey, 1985). Finally, *A. stolonifera* has been observed to hybridize with other *Agrostis* species, particularly *A. tenuis* Sibth. (Bradshaw, 1958a;

Davies, 1953). Morphology of the hybrids is variably intermediate between the two parent species (Bradshaw, 1958a) and may account for some of the confusion.

Hitchcock (1950, 1971) considers *A. palustris* (*A. stolonifera)* as a potential native species to northern North America. Meyer and Funk (1989) and Hanson *et al*. (1969) consider creeping bentgrass to be introduced to North America from Eurasia. The USDA, NRCS-Plants Database lists *A. stolonifera* as a native species (USDA, NRCS, 2001), perhaps based on Hitchcock's hypothesis (Hitchcock, 1950).

C. Genetics

The cytological work, although currently limited, may eventually prove more useful in determining species status. A cytological characterization of the bentgrass species was conducted by Keith Jones (1956a,b,c). His elegant work, although conducted in the 1950's, is still considered the standard reference on bentgrass genetics. He reports on the cytological characteristics of velvet bentgrass (*A. canina* var. *canina* and *A. canina* var. *montana*), colonial bentgrass (*A. tenuis,* currently recognized as *A. capillaris*), creeping bentgrass (*A. stolonifera* syn. *A. palustris*) and redtop (*A. gigantea*). Jones also characterized the cytology of *Agrostis* hybrids including, *A. canina* var. *canina* (currently recognized as A. *canina*) x *A. canina* var. *montana* (currently recognized as *A. vinealis*), *A. canina* var. *montana* x *A. tenuis*, *A. canina* var *montana* x *A. stolonifera*, *A. gigantea* x *A. tenuis* and *A. gigantea* x *A. stolonifera*. Chromosome counts were conducted on all species and hybrids (Table II.2). The basic chromosome number of all *Agrostis* species is x=7. Creeping bentgrass was found to be a strict allotetraploid with 2n=4x=28 with 14 bivalents forming at meiosis (Jones, 1956b; Church 1936). Jones (1956a,b,c) and Stebbins (1971) reported that the genus *Agrostis* might also have accessory or B chromosomes.

Warnke *et al.* (1998) confirmed the allotetraploid genomic constitution of 2n=28 for creeping bentgrass. The following genomic constitution of each species (or subspecies) was proposed by Jones (1956a,b,c) showing the currently recognized species designations:

Table II.2. Genomic constitution of several common bentgrass species (Jones 1956a,b,c)

Species	**Chromosome Number**	**Genome Constitution**
Velvet bentgrass (*A. canina*)	14	A_1A_1
Brown velvet bentgrass (*A.vinealis*)	28	$A_1A_1A_1A_1$
Colonial bentgrass (*A. capillaris*)	28	$A_1A_1A_2A_2$
Creeping bentgrass (*A.palustris, A. stolonifera*)	28	$A_2A_2A_3A_3$
Redtop (*A. gigantea*)	42	$A_1A_1A_2A_2A_3A_3$

Jones' idea for the cytological work arose due to significant taxonomic and cytological confusion concerning all of the species in the genus *Agrostis*. Work conducted prior to Jones mentioned numerous conflicting reports about the cytology and taxonomy of *Agrostis* species and putative interspecific hybrids. Differences in chromosome numbers within a species, differences in adaptation and occurrence of interspecific hybrids and differences in the possibilities and success of interspecific crosses beyond the F_1 generation are reported in the literature dating back to the late 1890s.

Jones (1956a,b,c) and Bradshaw (1958a) concluded that, while interspecific F1 hybrids were possible, genomic differences between species, incomplete homology between genomes, low seed set of F_1 hybrid plants and potential for functionally sterile aneuploid gametes and/or aneuploid plants would act as an effective barrier to gene exchange between species.

In possible support of the theories of Jones and Bradshaw, Bjorkman (1954) identified 600 tetraploids (2n=28), 160 pentaploids (2n=35) and 135 hexaploids (2n=42) from his 897-plant *A. stolonifera* collection. Only two aneuploid plants were recovered from his broad geographic collection of vegetative material. The hexaploid and pentaploid types produced almost exclusively aneuploid progeny (17/18 and 31/32 respectively) upon self-pollination. When he crossed hexaploid and tetraploid cytotypes artificially, he recovered 26 progeny and 25 of them were aneuploid. When he crossed pentaploid and tetraploid plants, he recovered 7 progeny, 6 of which were aneuploid. Jones (1956b) found it interesting to note that Bjorkman's field collections had been almost exclusively selected for euploid chromosome numbers in light of the high proportion of pentaploids in the original population and the proportion of aneuploid progeny. However, the artificial nature of Bjorkman's forced crosses and seedling establishment under noncompetitive greenhouse conditions does not mimic field conditions.

If Bjorkman's plants were interspecific hybrids, then the lack of aneuploids among the original collected parent plants with such a large proportion of 2n=35 types suggests that F_2 hybrids and/or F_1 backcrosses to the parent species are uncommon or are unlikely to persist in nature. Sterility of creeping bentgrass F_1 interspecific hybrids due to meiotic irregularity (demonstrated in *Agrostis* by Jones, 1956a,b,c and Bradshaw, 1958b) or poor fitness and survival of typically weak aneuploid F_2 and backcross plants are possible explanations for Bjorkman's results.

Bradshaw (1958a) studied the natural hybridization between *A. stolonifera* and *A. tenuis* (believed to be *A. capillaris* since it was the tetraploid form). Bradshaw (1958b) believed that the study area also contained a few putative F_2 and backcross hybrid plants based on plant morphological characteristics. There were varying degrees of fertility in the F_1, F_2 and backcross plants, with putative backcross plants having a higher degree of fertility. Bradshaw (1958a,b) also noted that his hybrids tended to be morphologically intermediate to the parent species, which makes absolute taxonomic diagnosis without cytology very difficult.

However, Kik *et al.* (1992) reported a similar range of ploidy levels within *A. stolonifera*. They later demonstrated both ploidy level and somatic chromosome number variation in individual plants of *A. stolonifera* (Kik *et al*., 1993). Therefore, another possible explanation for the lack of aneuploids in Bjorkman's population is chimeric development of tissues with different ploidy levels within *A. stolonifera.* The chimeras may eventually give rise to plantlets that become individual entities over time. However, Bjorkman (1954) may have been observing plants displaying Kik's aneusomaty in *A. stolonifera*.

Kik*, et al*. (1992) observed some relationship between ploidy level and ecological niche. Higher ploidy levels occurred with greater frequency in stable environments where clones could develop over a period of years and the role of sexual reproduction in survival was reduced. Non-tetraploids were not observed in highly unstable environments where high levels of individual turnover in the population relied on a seed bank for survival of a colony. Bradshaw (1958a,b) noted that the higher ploidy forms of creeping bentgrass are rare and almost wholly sterile which would tend to favor them in intensively grazed or undisturbed sites. Because *Agrostis* hybrid plant morphology (on which most of the taxonomy of the genera is based) is so similar to *A. stolonifera*, creeping bentgrass, the hybrids are difficult if not impossible to distinguish from creeping bentgrass. Therefore, it is possible that reports of higher ploidy level for *A. stolonifera* by Bjorkman and Kik may have been either observations of hybrid plants or cytotypes of *A. stolonifera* with varying ploidy levels.

Because of the similarity of bentgrasses and their hybrids in general and the lack of any reported or recorded serious impact of the hybrids, the study of *Agrostis* cytology and hybridization is more out of curiosity and an academic exercise rather than out of concern of their formation.

D. Related species

Other cultivated species of *Agrostis* include colonial bentgrass, redtop, velvet bentgrass, dryland bentgrass and Idaho redtop. Like creeping bentgrass, all of these species are perennial, wind-pollinated grasses that flower once per year and set seed during late summer. None of the cultivated, introduced or native *Agrostis* species are listed as noxious or serious weeds in United States agriculture, except in turf. None of the *Agrostis* species appear on the USDA, APHIS, PPQ, Federal Noxious Weed list as of June 7, 1999. Six states consider *Agrostis* species seeds "Noxious Weed Seed" or "Undesirable Weed Seed" in seed of other turfgrasses (USDA, 1997).

Cultivated *Agrostis* Species:
The *Agrostis* species used for turf culture have been selected for their ability to persist from one year to the next. These species are also selected to tolerate regular mowing at a low height of cut and regular traffic yet still provide a dense and uniform sward that can recuperate from wear. In addition, the species must be able to produce an economical seed yield for production, distribution and establishment.

D.1. Colonial bentgrass

Colonial bentgrass, *A. capillaris* (previously *A. tenuis*) (Hitchcock, 1950; Widen, 1971; McNeill and Dore, 1976), is generally regarded as having 28 chromosomes (Bjorkman, 1954; Sokolovskaya, 1938). Jones (1956b) identified colonial bentgrass as a segmental allotetraploid with one of its diploid ancestors in common with *A. stolonifera* and the other belonging to *A. canina*. Stuckey and Banfield (1946) reported an almost complete aneuploid series of 28 to 42 among seeds collected from pasture plants they first identified as *A. tenuis*. However, they do conclude that most of the 2n=42 plants resembled *A. alba,* even though they were first thought to be *A. tenuis*. Jones (1956b) is of the opinion that Stuckey and Banfield were observing hybrid material and that these seedling chromosome numbers have little bearing on what the chromosome number of their parent plants may have been. Stuckey and Banfield made no conclusion regarding the origin of the aneuploid plants.

Colonial bentgrass is predominantly cultivated for lawns and recreational turf. This species is more commonly used in Europe than in the United States. Colonial bentgrass characteristically has a lighter green color compared to creeping bentgrass and produces short rhizomes and stolons (Christians, 1998). Fewer than 10 colonial bentgrass commercial cultivars are eligible for certified seed production in Oregon (OSU, 1999).

D.2. Velvet bentgrass

Hitchcock (1950) lists velvet bentgrass as being introduced into the United States. This species, *A. canina* (previously *A. canina* subspecies *canina* or with *A. canina* subspecies *fascicularis* (Hubbard, 1984)) is a diploid (2n=14, x=7) that usually forms 7 bivalents at meiosis (Bjorkman, 1951; Jones, 1956a). Jones (1956a) believes that brown velvet bentgrass (*A. canina* L. ssp. *montana* (Hartm.) Hartm.) (now recognized as *A. vinealis*), is most likely an autotetraploid (2n=4x=28) possibly derived from chromosome duplication within or between ecotypes of the diploid subspecies *canina*.

Velvet bentgrass has been used on a very limited basis on putting greens in the Pacific Northwest and New England other than its inclusion in imported South German bentgrass seed mixtures. The species is stoloniferous with a slow and low growing habit with high shoot density. It is reported to be more tolerant of lower mowing heights, cold, heat, drought and shade than creeping bentgrass, but can produce excessive thatch when managed under high maintenance conditions. Velvet bentgrass is competitive at low clipping heights, however its slow spreading rate and low growth habit is easily out-competed in most natural environments. Velvet bentgrass has a narrow niche adaptation and has intensive maintenance requirements (Turgeon, 1985). Three commercial varieties (Kingstown, Vesper and SR7200) are eligible for Certified seed production in Oregon with fewer than 500 total acres in production (Oregon State University, 2001a).

D.3. Redtop

Redtop, *A. gigantea* (preferred to *A. alba L.*,), is a hexaploid (2n=6x=42) (Jones, 1956c). Jones also concluded that the hexaploid redtop shares some homology with both the tetraploids: creeping and colonial bentgrass (Jones, 1956c). Hitchcock (1950) lists redtop as a perennial species introduced into the United States. Redtop produces only rhizomes, no stolons (Christians, 1998).

During the 1940s and 1950s redtop was often used as a nurse grass for turf establishment. In seed mixtures, redtop seedlings would germinate quickly but would yield over time to the desired turfgrasses in the mixture such as Kentucky bluegrass and fine fescue. Redtop inclusion as a nurse grass in improved seed mixtures was replaced by improved perennial ryegrasses in the 1970s. The species is rarely used in the turf industry today. Redtop is considered to be a short-lived perennial in turf (Beard, 1973) however, persistent patches of unknown age of redtop have been observed in cool-season turf areas (Christians, 1998). One variety was eligible for Certified seed production in 2000 (Oregon State University, 1999). It is estimated that Certified redtop seed is currently produced on less than 500 acres in Oregon.

D.4. Dryland bentgrass

Dryland bentgrass, *A. castellana* Boiss. & Reut., is considered a hexaploid (2n=6x=42) (Darlington and Ammal, 1945). Reports of 2n=28 are also given for *A. castellana* (Bjorkman, 1954). 'Highland' dryland bentgrass, until recently, was treated as a cultivar of colonial bentgrass in the U.K. (Shildrick, 1976), but Highland is on the borderline of the morphological characters described for colonial bentgrass and is very distinctive in turfgrass performance (Shildrick, 1976). The features of Highland bentgrass are quite distinctive from other cultivars of colonial bentgrass and include vigorous spread by rhizomes, formation of aerial tillers under high mowing height, a prominent, jagged ligule twice the size of normal colonial bentgrass and similar in size to creeping bentgrass, blue-green leaf color, tall open growth habit and good winter color (Shildrick, 1976). Hitchcock (1950) lists Highland as an aberrant form of A. *tenuis* that may be a distinct species belonging to *A. castellana* based on seed and plant examination. The description of dryland bentgrass, particularly leaf color and heading records suggest that dryland bentgrass is very similar to Highland bentgrass (Shildrick, 1976).

The species has limited application in turf or forage due to its high susceptibility to several common turf diseases including *Rhizoctonia* brown patch; however; it is recognized for its heat and drought tolerance. There were 2815 acres of certified Highland bentgrass seed produced in Oregon in 2001 (Oregon State University, 2001a).

D.5. Idaho redtop

Idaho redtop or Idaho bentgrass, *A. idahoensis* (may be syn. with *A. clavata auct. non Trin.* and *A. borealis* Hartman var. *recta*.) (USDA, NRCS. 2001) is a perennial tetraploid (2n=4x=28). Furthermore, Idaho redtop is classified as non-creeping, without stolons or rhizomes. The species is native to the western U.S. and is adapted to wet meadows or bogs at medium to high elevations (Hitchcock, 1950).

One variety, GolfStar, establishes well in turf plots, but has a dull green color and an upright growth habit that is less attractive than creeping, colonial or velvet bentgrass. In New Jersey turf trials this variety has shown excellent resistance to dollar spot (Bonos, *et al.* 1998). GolfStar is eligible for Certified seed production in Oregon in 2001 with 87 acres planted on 3 farms (Oregon State University, 2001b). It is estimated that less than 500 acres of Idaho redtop are in production in Idaho.

D.6. Minor *Agrostis* species

Any significant level of hybridization or unexpected impact of potential hybrids among and within less common *Agrostis* species is unlikely. This conclusion is based on the limited number of literature citations for naturalized hybrids among the *Agrostis* species selected for widespread perennial turf cultivation and seed production. In addition, there have been no reports of adverse agricultural impact by any *Agrostis* hybrid documented in the literature.

Carlbom (1967) did an exhaustive survey of native *Agrostis* species of the western United States. More recently, MacBryde (2002, personal communication) compiled a list of 37 Agrostis species existing in the United States (Appendix III). Narrow adaptation and limited distribution of many of the *Agrostis* species listed in Appendix IV will make hybridization with *A. stolonifera* unlikely in seed production areas and in turf use. For example, Carlbom noted that *A. howellii*, *A. diegoensis* and *A. perennans* were all adversely affected by wilt when temperature exceeded 85^0F in his experimental garden in Corvallis thus, providing strong evidence of the narrow endemic adaptation of these *Agrostis* species. Roundup tolerance is unlikely to increase the frequency of hybridization that may already occur with *A. stolonifera*. Differences in chromosome number between many *Agrostis* species and creeping bentgrass will further limit F1 generation fertility and fecundity of any potential hybrids.

E. Life history

Life history refers to the patterns of establishment, growth, fecundity and survival in a given species or genotype (Kik *et al*., 1990a). An understanding of species life history can be useful for predicting invasiveness or weediness in a particular ecological niche (Barrett, 1988).

E.1. Introduction

Agrostis stolonifera is native to or introduced and naturalized in a variety of coastal, lowland and upland settings (Kik *et al*., 1990a,b) and is known to tolerate transient flooding (Davies and Singh, 1983). Even though a moist, fertile soil environment is a common characteristic of *A. stolonifera* habitat (Hunt *et al*., 1987), it also survives in sand dune environments with poorer water and nutrient retention characteristics (Kik, 1989). In those areas where *A. stolonifera* prevails despite a lack of high soil moisture, high humidity appears to play a significant role in its survival. When grown for golf course use and seed production, *A. stolonifera* receives generous applications of water and nitrogen to ensure its survival and recuperation under adverse conditions of foot and equipment traffic as well as heavy play (Beard, 2002; Meyer and Funk, 1989).

Agrostis stolonifera plant stands may originate from either seeds or stolon nodes. Sexual reproduction and a seed source are more important in unstable sand dune environments where a high degree of individual plant mortality is prevalent in the population (Kik *et al.*, 1990a,b). This phenomenon contrasts with very stable grasslands where a higher incidence of vegetative propagation was reported. New *A. stolonifera* seedling establishment has not been found to significantly contribute to stand augmentation in established vegetated areas (Lush, 1989; Jonsdottir, 1991; Bullock *et al*., 1994; Howe and Snaydon, 1986; Rossi, 1999; Eriksson, 1989).

E.2. Survival

Hunt *et al*. (1987) reported that *A. stolonifera* colonized and prospered in environments with a high degree of disturbance and very limited environmental stress. Disturbance in this context refers to bare soil, which increases the probability of direct and firm contact of a seed or node with soil. Soil disturbance in an *A. stolonifera* environment can result from any of the following: tillage in seed production or turfgrass establishment sites, sod removal, burrowing animals, livestock grazing damage, golf course traffic or club damage, soil introduction through turfgrass topdressing or alluvial deposition. In a favorable environment, Crick and Grime (1987) demonstrated that *A. stolonifera* can rapidly produce roots. Once established, the plants spread primarily through clonal expansion via stolons (Jonsdottir, 1991; Kik *et al*., 1990b). Low environmental (light, moisture, nutrition, air, temperature) stress allows for extensive root development, the accumulation of carbohydrate reserves, production of vegetative tillers and increased survivability during temporary stress periods. Areas of high disturbance but high environmental stress tend to be populated by small colonies with more reliance on the seed bank for survival than vegetative mechanisms (Kik *et al*., 1990a,b).

Several studies suggest that rapid leaf succession rate in newly emerged seedlings contributes to the successful establishment of *A. stolonifera* and its ability to compete with surrounding vegetation. In a controlled environment study, Cattani (2001) reported that the second leaf must fully expand for an *A. stolonifera* seedling to persist longer than 14 days after emergence. Since tillering in *A. stolonifera* is not initiated until after the third

leaf has at least partially emerged under non-competitive conditions (Cattani, 1999), the attainment of this stage is critical for persistence as it doubles the number of vegetative axes. Cattani (1999) also reported that reduced light duration was shown to lead to a delay in tillering until after the full emergence of the third leaf, thus further jeopardizing seedling survival in competitive environments.

Survival of newly germinated seedlings from a heterotrophic to an autotrophic state, which is critical for survival, is most successful during initial colonization of a newly disturbed site and (Whalley *et al*., 1966). Consequently, successful augmentation of *A. stolonifera* populations by seedlings has been reported to occur in situations optimal for germination and establishment to occur. These include either major disturbance events in which the seed to soil contact is high (Sheldrick *et al*., 1990) or when seed is introduced into turf maintained at very low clipping heights (Cattani, 2001).

Conversely, successful establishment can be limited in existing swards due to either insufficient disturbance or excessive competition from the existing population. Howe and Snaydon (1986); Jonsdottir (1991) and Bullock *et al.* (1994) noted that seedling recruitment into natural stands and pastures, respectively, was unsuccessful in augmenting *A. stolonifera* populations. Sweeney and Danneberger (1998) were unable to demonstrate with molecular markers the success from direct interseeding *A. stolonifera* into an existing *A. stolonifera* stand utilized for golf putting green turf. The difficulty in establishment of *Agrostis* seedlings may be a consequence of the extremely small seed (0.07 mg seed^{-1}) size. Small seed requires a long duration of disturbance (reduced competition) and low stress for establishment and survival after germination (Cattani, 2001). Small seed may also preclude direct contact with soil due to root competition from the existing vegetative stand (Kendrick and Danneberger, 2002).

E.3. Growth

A. stolonifera is perennial in nature and can exhibit aggressive stolon growth (Eriksson, 1989; Watschke, 1995) under favorable conditions. The species prefers areas with high disturbance but low environmental stress levels, e.g. good mineral nutrition and water availability (Hunt *et al*., 1987). Kik *et al*. (1990a) reported that vegetative tillering and subsequent vegetative colonization were favored by stable environments rather than unstable and/or environmentally stressful ones. Although, Kik *et al*. (1991) reported that *A. stolonifera* mortality occurred under three environments varying in the level of environmental stress, clonal survival was highest in the lowest stress environment.

Tillers arise from axillary buds that are most active in the late summer to early autumn time frame and may function vegetatively or reproductively according to environmental stimuli (Turgeon, 2002). Tillers are growth and expansion mechanisms. Attached tillers lead to the in-place expansion of the plant and stolons lead to the production of independently rooted daughter plants at stolon nodes (Cattani and Struik, 2001). Stolons generally develop as competition within the plant necessitates better light for growth (Cattani and Struik, 2001). Kik *et al*. (1992) reported that long-lived vegetative clones

developed significant numbers of vegetative tillers. These tillers produced more massive vegetative organs with a higher probability of survival than fertile individuals.

Seed producers enhance *A. stolonifera* reproductive tillering for seed production by planting in rows and removing top-growth after harvest. Golf courses manage *A. stolonifera* with frequent close mowing, aggressive cultivation, overseeding and sufficient agronomic inputs to achieve the highest shoot density and shortest internode length. Limited numbers of large, clonal colonies are less favored under these conditions than are large numbers of individual plants that contribute to visual and physical uniformity across the entire sward.

E.4. Fecundity

Fecundity refers to both reproductive and/or vegetative success of an organism in nature (Barbour, 1987). Both aspects of fecundity are important when considering *A. stolonifera* because of its predominantly clonal expansion activity in stable environments and seed production under environmental stress.

Collections of *A. stolonifera* from a sand dune with water and nutritional deficiencies were exclusively tetraploid and potentially fertile (Kik *et al.*, 1990b). In 1992, Kik *et al*. further reported that as the stability of the environment of origin increased, higher ploidy levels increased as well and produced more robust vegetative tillers. Ploidy levels exceeding four were previously reported to be highly infertile (Bradshaw, 1958a; Bjorkman, 1954; Kik *et al.*, 1992). These higher ploidy level individuals would rely on vegetative success rather than seed and seedling recruitment under low disturbance to survive. Aldrich (1984) notes that increased ploidy levels and fertility reduction are common to a wide range of perennial species in forage and other stable production systems.

Factors influencing reproductive *A. stolonifera* tillering include age of the tillers (Jonsdottir, 1991), environmental conditions (Turgeon, 2002) and cytotype of (Kik *et al*., 1992). Jonsdottir (1991) reported that tillers arising after July in a natural stand are more likely to produce inflorescences in the following year and that second season tillers were the most productive. Studies of seed production methods suggest that renovation and associated tiller stimulation increase yields and are most effective when they occur shortly after harvest (Cattani *et al*., 1997).

Seed yields for *A. stolonifera* vary with environmental conditions. *Agrostis* species are considered to have low seed bank populations in nature (Frame, 1990), which may be due to poor seed production and/or poor seed persistence. Pastures and golf course turfgrass favor vegetative propagation over sexual reproduction because the environment is usually stable and the reproductive organs are routinely removed by clipping (Lush, 1988a). Williams (1984) found clipping reduced *A. capillaris* seed bank presence by about 18% per year. Golf course superintendents attempt to maintain the seed bank and high shoot density with a program of routine overseeding and cultivation.

Pollen-mediated gene flow on golf courses is considered unlikely due to regular mowing and the low probability of seedhead formation when maintained at a low cutting height. This consensus was drawn from a diverse working group of scientists (including academics and industry representatives) regarding gene flow from herbicide resistant bentgrasses and bluegrasses (Quemada, 1999) and is consistent with the findings of Johnson and Riordan (1999) and Lush (1988a). However, Quemada reported the workgroup's conclusion that escape was inevitable, but that herbicide resistance was unlikely to make creeping bentgrass or other turf species more invasive since they are "ill-adapted" for unmanaged ecosystems.

Vegetative expansion of *A. stolonifera* is related to carbohydrate storage in vegetative tillers (Kik *et al.*, 1992). Studies of *A. stolonifera* forage productivity in a monoculture (Haggar, 1976) and in a mixed stand (Frame, 1990) with white clover (*Trifolium repens* L.) reported that dry matter production is less than that of most other common forage grasses. While the volunteer occurrence of *A. stolonifera* colonies in pasture and some unmanaged settings is testimony to its vegetative success, its preference for generous water and nutrients limits that success in mixed populations to well-defined habitats.

E.5. Seed viability

The minimum germination percentage for Oregon Certified creeping bentgrass seed under the Association of Official Seed Analysts (AOSA, 1998) standard germination test is 85%. In addition, there appears to be some post-harvest ripening requirement, as the AOSCA (2001) requires a 7-day chilling before germination testing.

Seed persistence data from controlled laboratory or field research is not common. Cattani (pers. comm., 2002) has observed volunteer germination of *A. stolonifera* when sod was stripped from a putting green five years after initial establishment. Bekker *et al*. (2000) estimated seed persistence for *Agrostis spp.* as long as 25 years.

Seed production takes place early in the overall *Agrostis spp.* life span. Bekker *et al*. (2000) report that *Agrostis spp.* add little seed to the seed bank after the first few years of growth and seed bank presence is found primarily below the 5 cm level later in stand life. This finding suggests that seeds above 5 cm germinate and then either contribute to the establishment of an initial sward or succumb to competition from mature grasses in established swards. Seeds below 5 cm are apparently not stimulated to germinate.

E.6. Pollen movement

Wipff and Fricker (2001) measured bentgrass pollen traveling up to 292 m (958 feet) from the source during 1999 where they recovered a single plant among more than a thousand with the transgene. Wipff and Fricker (2001) modeled their 1998 and 1999 results. The best R^2 values from these models predicted transgene flows of 0.1% at 808 ft and 0.02% at 1,022 feet from 1999 data. R^2 values of data fit from these regression models were 0.1781 to 0.3817 in 1998 and 0.4516 to 0.8662 in 1999. The practical significance of their

observations is unclear given that pedigreed seed isolation distances in place for decades have been sufficient to maintain accepted purity standards assigned to the various seed classes.

Belanger *et al.* (2003) examined intraspecific glufosinate tolerant transgene flow and interspecific transgene flow to non-transgenic *A. stolonifera, A. capillaris*, *A. castellana*, *A. gigantea* and *A. canina.* One plant of each species was planted at each sample point and 3 meters separated each sample point. The maximum distance from the centrally located transgenic source plants was 15 meters. The frequencies of transgenic hybrid recovery for *A. capillaris*, *A. castellana*, *A. gigantea* and *A. canina* within 15 meters in all directions were 0.044% (44,967 seedlings screened), 0.0015% (663,778 seedlings screened), 0.0% (2,298,418 seedlings screened) and 0.0% (7,556 seedlings screened), respectively for interspecific flow. Frequency of recovery for intra-specific transgene flow was 0.631% (155,773 seedlings screened). They further stated that although transgene flow occurs, the herbicide resistant transgenes would be unlikely to confer any competitive advantage to *Agrostis spp.* in natural ecosystems where herbicides are unlikely to be used.

It is important to note that pollen-mediated gene flow experiments conducted with isolated receptor plants (e.g., Wipff and Fricker, 2001; Belanger *et al*. 2003) will have a tendency to overestimate the amount of pollen movement with inter-varietal crossing under production field conditions since isolated or small populations of pollen receptor plants are more apt to be pollinated by their nearest neighbor than a remote pollen source. Consequently, pollination from distant source plants will be significantly less than in a non-competitive situation as used in this experiment. A number of factors will impact cross fertilization between grasses. These include: (1) synchrony of pollen shed and receptive stigmas (date and time of day); (2) proximity of the plants; (3) wind speed and direction; (4) pollen viability and longevity; (5) temperature and relative humidity and (6) compatibility between pollen and stigmas or styles (Burton, 1992).

Davies (1953) recorded anthesis dates and time of day for pollen shed in several *Agrostis* species from the British Isles (Table II.3). Whereas anthesis dates did overlap, it is apparent that there may be some isolation from pollen-mediated gene flow between some species provided by the pollination time of day. Belanger *et al.* (2003) were able to force hybridization in the greenhouse between *A. stolonifera* and *A. gigantea* or *A. canina* by artificially synchronizing anthesis and bagging panicles together, but they did not recover the hybrids in field studies conducted over two years. Belanger's group concluded that differences in anthesis dates for *Agrostis spp.* may limit gene flow between them.

Table II.3. Time of day for pollen shed in several *Agrostis* species (Davies, 1953)

Agrostis spp.	**Pollen shed**
A. stolonifera	10:00 – 11:30 am
A. tenuis (*A. capillaris*)	1:00 – 5:00 pm
A. gigantea	2:30 – 3:30 pm
A. canina var. *arida*	4:00 – 5:00 am
A. canina var. *fascicularis*	4:30 – 5:30 am

Size of the plant population and planting density have a major impact on outcrossing frequency due to intra-varietal pollen competition for receptive stigmas. In a series of pollination studies designed to examine isolation distance and competing pollen sources in perennial ryegrass, Griffiths (1951) concluded that the effects of intra-varietal (within a single field) pollen competition were highly effective in reducing inter-varietal crossing (between fields) and were similar to the effects of distance. Pollen contamination decreases rapidly with distance. However, over short distances, intra-varietal pollen competition was more effective than distance because the nearest neighboring plants are likely to provide the biggest pollen contribution. These results help provide the basis for Griffiths' recommendation for (and the currently common practice of) cutting border rows from large Certified fields after pollination where adequate isolation cannot be provided by distance. Similar conclusions were drawn by Heribert-Nilsson as cited in Griffiths (1951) for rye, by Knowles (1966) in smooth bromegrass and by Copeland and Hardin (1970) in perennial ryegrass.

Isolation distances prescribed for pedigree creeping bentgrass seed production vary depending on the class of Certified seed and the size of the field (Oregon State University, 2001a). Certified fields of less than 5 acres require 300 feet isolation from other *Agrostis spp.* while fields of more than 5 acres require only 165 feet isolation. Isolation distance required for Foundation seed fields of any size is 900 feet. The percentage of observable other-variety or off-type plants permitted is 2% in a Certified class eligible seed production field and 0.1% in a Registered class eligible seed production field. No visible off-type plants are permitted in a Foundation class eligible seed production field. These maximum thresholds only apply to the plants that are present in the field producing the particular class of Certified seed.

Jones and Newell (1946) confirmed the efficacy of distance isolation by studying the distribution of pollen at distance from source populations of several grass species. They found that the average amount of pollen captured at 990' from the source was <1% of that found at the source, however, there was no attempt to measure the viability of the pollen. They attributed the rapid decline in pollen concentration to gravity and dispersion. Pollen competition from adjacent fields of the same species would further reduce the potential that this pollen would find unfertilized and receptive stigmas.

E.6.a. Pollen viability

No specific literature reference documents the pollen viability characteristics of *A. stolonifera.* The Environmental Assessment of Pure Seed Testing's USDA APHIS Field Release Permit (97-087-02r) for glufosinate-tolerant / putative disease resistant bentgrass (Koehler, 1997) cites a pollen viability period of three hours but does not supply a reference. This assumption is most likely drawn from data on other species. Teare *et al*. (1970) determined that 98.6% of Kentucky bluegrass pollen began losing viability within three hours between 6 AM and 9 AM, as temperature was increasing and relative humidity was decreasing. No pollen was viable after 11 AM. They believed that factors other than temperature and humidity may be important for Kentucky bluegrass pollen longevity, but did not elaborate on what those may be. Knowlton (1922) determined that desiccation is the normal cause of death in corn pollen. Jones and Newell (1948) concluded that low temperatures and high relative humidity were best for maintaining grass pollen longevity. These studies suggest that pollen longevity is probably greatest in cool and humid environments. Weather conditions during *A. stolonifera* pollination in the Willamette Valley or other inland locations are typically hot and dry, and should rapidly reduce pollen viability over time and distance. These adverse climatic conditions contribute to reducing the potential for intra- or inter- specific hybridization.

E.6.b. Hybridization with other species

E.6.b.1. Interspecific crossing

There have been varying reports of interspecific crossing within the genus *Agrostis* (Davies, 1953; Jones 1956a,b,c; Bradshaw, 1958a; Hegi, 1935; Tutin, 1980; Welsh *et al*. 1987; Wipff and Fricker, 2001; Belanger, 2003). However, many of these hybrids were artificially produced or unconfirmed field diagnoses. The hybrids described by Davies, Jones and Bradshaw were usually morphologically intermediate between the two parents and had a complete loss or much reduced fertility (Bradshaw, 1958a; Jones, 1956a,b,c). Differences were also noted for cytological characteristics of interspecific crossing. Finally, there is incomplete agreement among researchers regarding the genetic origin of plants with increased ploidy level, which have been attributed to both intra- and interspecific hybridization and intraplant/intraclone mutations.

Early assumptions of putative interspecific hybridization (Davies, 1953; Jones 1956abc; Bradshaw, 1958a) that were based on increased ploidy levels may be questionable in light of Kik *et al*. (1989, 1990b, 1992, 1993). They found that *A. stolonifera* clones collected from divergent ecosystems had differing cytological make-up including ploidy levels and related distributions of fertility. Kik *et al*. (1993) reported on within-plant somaclonal ploidy level variation and suggests this as a source of increased reproductive ploidy levels and associated sterility. This finding is an alternative interpretation for earlier assumptions of the link between interspecific crossing and elevated ploidy level.

E.6.b.2. Intergeneric hybridization

Like *Agrostis*, most *Polypogon spp.* have similar niche adaptation and limited distribution. Putative hybrids have been reported between *Agrostis* and some *Polypogon* species (Bjorkman,1960; Parodi, 1951; Welsh *et al*., 1987). Bjorkman (1954) states that *Polypogon* species are often placed in the genus *Agrostis* (ex. *P. semiverticillatus* (Forskal) Hyl. , syn. *A. verticillata* Villars) as they are very similar in appearance and adaptation. Sokolavskaya (1938) believes that *P. litoralis* is synonymous with *A. verticillata*. Bjorkman (1960) mentions sterile putative hybrids between *A. stolonifera* and *P. fugax* or *P. semiverticillatus* (syn. *A. verticillata* Villars). He also notes (Bjorkman, 1954) the species *P. fugax* Nees. is *P. litoralis* Sm. (a name that was originally given to the hybrid *A. stolonifera* x *P. monspeliensis* L. Desf.). However, Sokolovskaya (1938) mentions that early researchers felt that *P. litoralis* is a cross between *P. monspeliensis* and *A. alba*. Bjorkman (1960) later states that hybrids resulting from *A. stolonifera* crosses with *P. monspeliensis* L. are sterile.
Hitchcock (1950) lists other *Polypogon* species that may be found in the U.S. including:

- *P. interruptus* HBK, Ditch Polypogon. This perennial tufted non-creeping species has a limited adaptation to wet areas and ditches and would be of limited threat to agriculture. The species has been reported in CA, OR, WA and east to LA, NE and OK.
- *P. australis* Brongn. is listed as a perennial introduced in WA.
- *P. maritimus* Willd. is listed as an introduced annual occurring in GA, NE, CA.
- 2n = 14, 28.
- *P. elongatus* HBK is a perennial found in wet places in AZ.

Confusion in distinguishing *Polypogon* and *Agrostis* is widespread and there is disagreement on the relationships (Sokolovskaya, 1938). On the rare occasions that hybrids have been noted, the hybrids have been sterile (Bjorkman, 1960).

Koehler (1997) further summarized the impact and potential for *A. stolonifera* to form inter-generic hybrids. The following is extracted from the Environmental Assessment performed to approve USDA permit number 97-087-02r for glufosinate-tolerant / putative disease resistant bentgrass:

"Intergeneric hybrids are known to naturally occur between A. stolonifera and Polypogon monspeliensis (L) Desf., but the hybrids are sterile (Björkman,1960). Sterile hybrids have also been obtained with P. fugax and P. semiverticillatus (= P. viridis) (Björkman,1960). Many of these Polypogon species have a high degree of self-fertility, which would not favor the formation of hybrids in nature. P. monspeliensis has been described as a common weed in the western U.S. (Hitchcock, 1935). P. interruptus H. B. K (Chase, 1950), described as existing in ditches and wet places at low altitudes including from British Columbia to California, is listed as a weed of unspecified status in the continental U.S. by Holm et al. (1979). Based on its similarity to P. fugax as reported by Björkman (1960), it is unlikely to form fertile hybrids with A. stolonifera."

The USDA concluded that inter-generic hybrids between *Agrostis* and *Polypogon* are likely to be infrequent, sterile and of no significant impact (Koehler, 1997).

E.6.b.2.a. F1 survivability and growth

Bradshaw (1958b) concluded that the sterility of the F_1 hybrid between *A. tenuis* Sibth. and *A. stolonifera* L. is not important to its persistence in the environment in which it arose because it is well-adapted vegetatively. Sterile or partly sterile vegetative F1 hybrid clones would spread and persist only in conditions of high disturbance and low environmental stress. Perennial success of a colony should decline during periods when these conditions are not met. This suggests that the long-term survivability of sterile clones is limited in nature. This notion is supported by the ploidy levels reported in Kik *et al.* (1993), where fertile tetraploids ranged from 23-100% of the population regardless of the ecological setting.

E.6.b.2.b. F1 fertility

Most researchers have referred to sterility as a defining characteristic of interspecific hybrids and have attributed the phenomenon to reduced genome homology between parents that leads to sterile gametes or aneuploidy among F2 progeny (Jones, 1956a,b,c). While fertile hybrid crosses have occasionally been reported, they remain rare in the literature.

F. Weediness of *A. stolonifera*

F.1. Weed development potential

Weeds may be characterized in various ways, the most simplistic and subjective being "a plant out of place." In practice, weeds tend to carry an economic cost related to crop production or environmental management (Hartwig, 1996; Zimdahl, 1999; Aldrich, 1984). The economic perspective implies that while *A. stolonifera* may be undesirable in certain locations, only host sites with sufficient economic or social value will warrant the expense and/or effort to manage it as an economic pest. In this context, *A. stolonifera* may be considered an economic weed in a limited number of circumstances: (1) as an escape from and/or volunteer in seed production fields in regions of diverse grass species production for pedigreed seed, (2) when growing or spreading into areas planted to other turfgrass types, (3) a pest of food or fiber crops, or (4) a colonizer of nonagricultural habitats possessing exceptional value to society and the environment.

F.2. Characteristics of weedy and invasive species

Holt (1988) reviewed the characteristics of weed success as opposed to evolutionary success because agricultural practice does not mimic evolution in nature. She identifies three components of weed success: rapid colonization of disturbed sites, difficulty in

removal and suppression of crop plants. The first two measures are functions of a plant's adaptive strategy while the third relates to its competitiveness.

In general, for weeds to succeed in a given ecological niche they must exhibit advantageous characteristics that include survivability, growth and reproductive fitness. The ecological niche, in this case, is typically defined by an agricultural production system rather than nature. Holt adapts a stress-disturbance model to her discussion of strategic adaptation. She argues that weeds can succeed based on their adaptation to system disturbances, such as tillage-induced germination or to environmental stresses, which include summer dormancy of cool season grasses, but not to both. Disturbance-tolerators thrive on disturbance in the absence of stress and are typically annuals with high seed production and rapid life cycles. Environmental stress-tolerators are most typically perennials in undisturbed areas. Species that tolerate neither disturbance nor stress are competitors because of their success in populating the most desirable and competitive ecological niche. Booth and Swanton (2002) note that selection within an environment is for characteristics that are required for survival. The authors also concluded that herbicide tolerance does not confer (and may take a way from) the ability to out-compete other species in the absence of the herbicide.

Many of the worst agricultural weeds (Holm *et al.*, 1979) are disturbance-tolerators because annual cropping is prevalent over perennial plants in most settings (Janick *et al.*, 1974). In annual cropping, system disturbance is high (tillage, cultivation, traffic) and environmental stress is minimized (water, nutrient, pH management), favoring strong germinators and fast maturing individuals (Zimdahl, 1999; Aldrich 1984). Many perennials in nature thrive on minimal system disturbance so they can build extensive water and nutrient collecting systems for survival under adverse environmental conditions. However, perennials that are stress-tolerators in nature can behave as disturbance-tolerators in agricultural systems if water and nutrient inputs are sufficient to offset the effects of system disturbances (Aldrich, 1984), e.g., johnsongrass in soybean and cotton culture in the south. This is especially true of vegetatively propagating colonizers that survive or benefit from mechanical fragmentation, which mimics seed as a dispersal mechanism for increasing population numbers.

Perennial weeds tend to be more significant pests in perennial crops than annuals, except for herbaceous species with fecund vegetative propagules (Ross and Lembi, 1999). The most successful examples store large carbohydrate reserves that provide regenerative capacity under field conditions or permit long dormancy, enable repeated recovery from defoliation and are stimulated by mechanical fragmentation.

F.3. General status of *A. stolonifera* as a weed

As noted previously, *A. stolonifera* thrives in moist, nutrient-rich habitats and is found naturally in pastures and meadows, stream and ditch banks and coastal environments. While observed in less ideal habitats, the species does not thrive in these instances and is

not particularly competitive with better-adapted species (Crick and Grime, 1987; Haggar, 1976; Marshall, 1990; Smith, 1972).

A. stolonifera is not listed as a serious, principal or common weed in the continental U.S. by Holm *et al*. (1979). In addition, *Agrostis* species are not listed in Weeds of the Northeast (Uva *et al.,* 1997), although they are known to occur in the wild there. None of the *Agrostis* species appears on the USDA Federal Noxious Weed list as of September 8, 2001 (USDA, 2002a).

F.3.a. *A. stolonifera* as a weed in grass seed production

Agrostis stolonifera seed has been produced in the Willamette Valley of Oregon for more than 75 years (Schoth, 1930). In general, cross contamination of grass seed with off-types in fields and post-harvest processing operations has been minimized by the seed certification standards of the Oregon Seed Certification Service. All volunteer grasses, including *A. stolonifera,* are considered weeds when they occur in production fields of other grass types and are highly undesirable due to the negative impact on crop value in light of certification standards. While the plants are not regulated weeds, six states include seeds of *Agrostis* species along with tall fescue, bermudagrass, orchardgrass, and timothy in a list of "Noxious Weed Seed" or "Undesirable Grass Seed, " if they occur in seed of other turf grasses (USDA, 2002b).

Assuming some limited establishment in grass seed production fields, *A. stolonifera* exhibits a number of inherent characteristics that minimize its potential to contaminate harvested seed of other grass types:

- *A. stolonifera* develops at a slower rate than other grasses and requires an earlier fall planting date compared to most other turfgrass species. *A. stolonifera* that germinates with another commercial species is less likely to mature physically for winter survival, to exhibit floral induction and initiation the following spring or to successfully produce seed.

- *A. stolonifera* is the last grass to mature in the Willamette Valley of Oregon and therefore the last harvested, usually in late July or August. *Lolium perrene*, *Lolium multiflorum, Festuca arundinacea, Festuca longifolia and Festuca rubra spp.* are each harvested 3 to 5 weeks earlier, when most *A. stolonifera* seed is still immature. Thus, *A. stolonifera* seed is unlikely to be viable even when it is present as a contaminant.

- *A.stolonifera* is a shallow rooted species that requires more consistent soil moisture than other species in order to persist and produce seed. Summers are very dry in the Willamette Valley of Oregon and supplemental irrigation is required for the production of viable *A. stolonifera* seed. Other grasses typically are produced without irrigation, so *A. stolonifera* do not thrive in their midst (Meyer and Funk, 1989).

- *A. stolonifera* seed is approximately 1/20 the size of *Lolium multiflorum, L. perenne, Festuca arundinaceae, and F. rubra*. Most *A. stolonifera* seed that might contaminate seed of these other species is removed routinely in combines and various seed cleaners in downstream conditioning operations. It is not listed as a common contaminant of grass seed by the OSCS (Oregon State University, 2001a).

These facts are confirmed by reports listing the top 10 most frequently found contaminants of grass seeds identified during routine seed purity tests. *A. stolonifera* is consistently absent from these reports indicating that it is not persisting in fields of other grass species. The tenth most frequently found contaminant is found in about 1 in 100 seed lots. (Oregon State University, 2001a.).

F.3.b. *A. stolonifera* as a weed in managed turfgrass

Agrostis stolonifera has been grown in the U.S. on golf putting greens for over 100 years and for over 50 years for fairways and has never been considered an unusually serious weed of other turfgrasses. When it is considered a weed of other turfgrass species, it is due to the combination of its creeping habit and unique texture that combine to create non-uniform patches that deteriorate in quality and aesthetics when mowed above one-half inch (Watschke, 1995). The most common scenarios are lateral growth of *A. stolonifera* off of golf putting greens into surrounding turfgrass and the contamination of other turfgrass seed that leads to patches in residential and commercial lawns. Nonetheless, *A. stolonifera* rarely earns the attention of professional turfgrass managers to the extent of crabgrass or dandelion because it is a relatively uncommon and localized concern.

F.3.c. *A. stolonifera* occurrence in general agriculture

None of the cultivated or native *Agrostis* species are listed as noxious or serious weeds in U.S. agriculture, except in turfgrass swards and seed production fields of other grass species (Holm *et al.*, 1979). Tillage operations, herbicide programs and economical water management in most systems prohibit establishment and spread of the species. No mention of *A. stolonifera* is made in three prominent weed science texts (Zimdahl, 1999; Ross and Lembi, 1999; Aldrich, 1984)

A. stolonifera does occur in pastures of moist and humid environments in the temperate zones. However, it has been naturally occurring in many pastures for centuries and is not necessarily considered a weed there (Bradshaw, 1958a). It is, in fact, one of the few cool season grass species that can tolerate frequent and continuous grazing because of its low growing and stoloniferous growth habit. Frame (1990) and Haggar (1976) both note that *A. stolonifera* is often less productive than other forages and may be less desirable for this reason, but it is seldom referred to as a problem weed.

F.3.d. *A. stolonifera* in natural systems

A. stolonifera has been introduced to and naturalized in many locales around the world. The species has not developed into an ecological problem in the vast majority of them. In one very unusual occurrence, Gremmen *et al*. (1998) reported on the remarkable colonization and competitive success of *A. stolonifera* introduced in the 1950s to sub-Antarctic Marion Island. In this isolated occurrence the species established in mesic to wet habitats on Marion Island and became predominant in these niches.

F.4. Weed performance and economic aspects

Generally, *A. stolonifera* has exhibited better success at establishing and colonizing newly disturbed sites than at expansion in stable environments (Sweeney and Danneberger, 1998; Jonsdottir, 1991). Seedling establishment is only marginally successful in highly competitive environments. Lush (1988a) found no *A. stolonifera* seedlings in an *A. stolonifera/P. annua* turf even though *A. stolonifera* seed was present in the seed bank (Lush 1988b). Vegetative colonization is most effective when moisture and disturbance conditions are optimal and competition is reduced.

Sweeney and Danneberger (1998) observed very low *A. stolonifera* establishment rates based on DNA analysis of 28 plants after four consecutive years of inter-seeding with core aeration into an *A. stolonifera* putting green. Howe and Snaydon (1986) reported less than 5% of the *A. stolonifera* seed sown directly into perennial rye grass pasture survived, regardless of nutrition level or season of establishment. This is in comparison to the much larger-seeded *Festuca rubra*, which exhibited seedling survival of up to 38.5%.

The difficulty in seedling establishment may be a consequence of the extremely small seed size (0.07 mg seed^{-1}) of *A. stolonifera*, which restricts establishment to a relatively short period after germination. Efficient transition from a heterotrophic to an autotrophic state is critical (Whalley *et al*., 1966) and may be prolonged in competitive settings. Bernston and Wayne (2000) reported that while below ground competition is linearly related to the size of the root system, above ground competition was asymmetric with respect to light capture, with plant height and leaf area being important positive factors. Plant height, in particular, is an obvious advantage of the existing vegetation over newly emerging vegetation (Bernston and Wayne, 2000). Jonsdottir (1991) and Bullock *et al*. (1994) both worked with canopies above 5 cm when unsuccessfully attempting to recruit seedlings in natural stands and pastures. Successful interseeding typically requires aggressive scalping of existing turf (Cattani and Struik, 2001; Kendrick and Danneberger, 1998; Sweeney and Danneberger, 1998).

Established seedlings will expand laterally through stolons, which root and form daughter plants at the nodes where moisture conditions are favorable for rooting. Attached daughter plants are less sensitive than seedlings to competition for light, nutrients and water during establishment because they derive resources from the mother plant (Ross and Lembi, 1999). However, observations from pastures and golf course putting greens

describe colonies of limited size and suggest that vegetative colonization is also sensitive to competition and tends to be successful in localized areas of ideal conditions (Lush, 1988a). *Agrostis stolonifera* is late to green up and mature among forage grasses and may suffer an early season disadvantage in competing for resources and expanding geographically (Haggar, 1976).

Once established, *A. stolonifera* resists competition effectively and colonies in mixed swards are long-lived. Effective competitors tend to be aggressive disturbance-tolerators adapted to the same environment such as annual bluegrass or more aggressive creeping species such as bermudagrass (Aldrich, 1984; Ross and Lembi, 1999).

F.4.a. Economic weediness of *A. stolonifera* in grass seed production

The potential for *A. stolonifera* to become a serious weed of other grass species grown for seed is limited not only by certification-driven field management practices but also by differences in crop production conditions and practices.

Agrostis stolonifera plants are most likely to be introduced into other grass seed production fields either through lapses in field sanitation procedures, e.g., viable stolons transported on tillage equipment, or as contaminated stock seed. Its creeping habit does encourage lateral growth, but border plants are removed as part of routine field sanitation. Successful establishment of *A. stolonifera* by either seed or stolon in seed fields of other grasses is discouraged because it requires high soil moisture or humidity to survive, and most other grass seeds in the region are grown under arid summer conditions without irrigation. Dryland production of these other grasses is facilitated by their larger seed size, which provides a larger carbohydrate reserve for initial establishment, deeper root system to tap deep soil moisture, and earlier maturity for harvest before soil moisture becomes limiting. Introduced *A. stolonifera* stolons are unlikely to root under dryland conditions and its very small seed size provides little energy for establishment under drought stress. Similarly, an occasional bentgrass seed germinating among a high population of large seeded grasses would be less fit to successfully compete for resources.

A stolonifera establishment from seed in other grass seed production fields would be further discouraged because of the widespread use of soil active herbicides used to control germinating seedlings (Oregon State University, 2001b). Turfgrass seed production fields are routinely treated with soil active, long residual herbicides such as pronamide, diuron, ethofumesate and others to control a range of grassy and broadleaf seedlings.

As noted in previous sections, *A. stolonifera* that manages to colonize production fields of other grass species is unlikely to produce viable seed due to differences in maturity timings, existing herbicide programs and harvest schedules. *Agrostis stolonifera* matures later than other grasses grown in the region and so will often be immature if harvested with other grasses. Dryland seed production systems should favor seed production over vegetative propagation of *A. stolonifera* (Kik *et al*., 1990a; Kik *et al.,* 1992), so

unsuccessful seed production should constitute the bulk of *A. stolonifera* activity and discourage aggressive vegetative expansion in the field.

Seed production fields are routinely scouted for genetic off-types, broadleaf and grassy weeds in keeping with certification standards. Hand or chemical roguing eliminates unwanted plants. This level of management intensity makes an uncontrollable or economically devastating infestation of *A. stolonifera* highly unlikely unless certification practices are not followed. *Agrostis stolonifera* possesses no unique competitive characteristics in the presence of other seed grasses that would constitute an overwhelming advantage and result in serious crop loss. In a worst case scenario, small areas of crop might be lost to localized herbicide application or tillage operations to eliminate problem colonies, but large scale infestations are very unusual. Control of *Agrostis sp.* in a turfgrass seed production field can be achieved with a variety of chemicals geared for seedling control. Crop rotation is also part of an integrated weed control program and contributes to control of volunteer *Agrostis* plants (Glenn Jacklin, 2002. personal communication, Appendix VIII).

F.4.b. Economic weediness of *A. stolonifera* in managed turfgrass

The most likely mechanisms of weedy *A. stolonifera* establishment in other turfgrasses are lateral encroachment from golf putting green or fairway cultures, escape from a previous golf course renovation or as a seed contaminant in new plantings (Beard, 2002; Dernoeden, 2002; Oregon State University, 2001a). Inputs to the turfgrass, such as water and nutrients, can favor the establishment and initial expansion of *A. stolonifera* in both golf course and seed production scenarios even in the face of competition from desirable grasses. *Agrostis stolonifera* is grown in a concentrated geographic area relative to total U.S. grass seed production (Oregon Department of Agriculture, 2001). The small seed size of *Agrostis* facilitates exclusion from larger seeds in processing so bentgrass-contaminated turfgrass seed is unusual. Oregon Seed Certification Service (Oregon State University, 2001a) does not identify *A. stolonifera* as among the top 10 weed contaminants of other turfgrass seed.

Seed is rarely a source of contamination by *A. stolonifera* in established stands of other turfgrasses. Numerous researchers have reported on the difficulty of successful seedling establishment in mature turfgrass stands, presumably due to competitive barriers that were explained earlier in this Section. Canopy height and resource competition appear to be critical issues. Lush (1988a) was unsuccessful in finding seedlings of *A. stolonifera* in a golf course putting green study managed with a cutting height of 6 mm (0.24 inches), even though *Poa annua* seedlings established readily. Little success was also reported from interseeding *A. stolonifera* into an existing *A. stolonifera* stand utilized for golf course putting green turf (Sweeney and Danneberger, 1998). The presence of *A. stolonifera* seed occurs almost exclusively through overseeding since natural *A. stolonifera* seedbank populations are low (Lush, 1988b) and cannot occur under typical mowing regimes because reproductive tillers are routinely removed (Lush, 1988a). Therefore, natural seed

dispersal from *A. stolonifera* stands to adjacent turfgrasses is essentially of no practical concern.

Vegetative invasion from golf course putting green or fairway *A. stolonifera* stands is generally limited to short distances from well-defined edges because of competition from existing stands (Beard, 2002). The impact is almost purely aesthetic. Establishment typically occurs when growth conditions favor *A. stolonifera* over the adjacent species. In southern climates where *A. stolonifera* may be grown on golf course putting greens next to warm season grass fairways, encroachment is more typically from bermudagrass or zoysiagrass fairways into *A. stolonifera* greens (Dernoeden, 2002). Established colonies of *A. stolonifera* are durable and long-lived but do not necessarily spread over large areas.

F.4.c. *A. stolonifera* weediness in general agriculture

Agrostis stolonifera infestations in annual or perennial food or fiber crops are not routinely reported, presumably because standard integrated weed control programs are completely effective at disrupting the plant life cycle and/or eliminating it. System disturbances associated with cropping systems such as mechanical tillage might seem to favor the establishment of *A. stolonifera.* Further the supplemental water needed by *A. stolonifera* is not provided in dry land management, which removes the ability of bentgrass to establish and persist. In perennial systems such as orchards, *A. stolonifera* is not differentiated from other sod-forming species because it poses no unique risk to orchard crops.

A. stolonifera has been demonstrated to establish in disturbed and abandoned agricultural fields and in pastures. However, in the latter, *A. stolonifera* is not necessarily desired due to its relative invasiveness (Schulte, 2001), low dry matter production, poor nitrogen use efficiency (Sheldrick *et al*., 1990) and low digestibility (Frame 1990). However, on continuously and intensively grazed areas of low stress, bentgrass is competitive (Bradshaw, 1958a). The invasiveness observed by Schulte (2001) resulted from a feeding preference for other grass species, which enabled *A. stolonifera* to persist. However, Jonsdottir (1991) and Bullock *et al.* (1994) noted that seedling recruitment into natural stands and pastures, respectively, was unsuccessful in augmenting *A. stolonifera* populations. In general, *A. stolonifera* is recognized as a colonizer of pastures but not with serious economic consequences.

F.4.d. *A. stolonifera* invasiveness in natural systems

Much of the biological information that is pertinent to invasiveness in natural systems was presented earlier in this Section. That information is used here to characterize invasiveness in natural systems directly.

It is difficult to predict where commercial seed might be introduced but the literature clearly indicates that introduced *A. stolonifera* colonizes and naturalizes in habitats similar to its origin, i.e., moist and/or humid meadows and early succession forests, but not widely

beyond these habitats (Collett *et al*., 1996). Further, since *Agrostis* species are considered to have low seed bank populations (Frame, 1990), the most likely means of introduction in a natural system outside of the geographic origin of *A. stolonifera* is through commercial seed (Gremmen *et al*., 1998). Stolon introduction could occur as a stowaway on tillage or excavating equipment but is highly unlikely in an isolated ecosystem. There are no reports of ecosystem disturbance by *A. stolonifera* outside of these conditions.

As discussed previously, Hunt *et al*. (1987) reported that *A. stolonifera* prefers environments with a high degree of disturbance and very limited environmental stress. Such environments are rarely unpopulated or noncompetitive in nature. In fact, these are exactly the conditions preferred by highly aggressive disturbance-tolerating species. This suggests that *A. stolonifera* would be unable to monopolize sites in the presence of significant competition from other plant species. Slow recovery from winter dormancy in the spring (Collet *et al*., 1996) is a competitive disadvantage, which is likely responsible for limiting *A. stolonifera* colonization in most natural settings.

The unusual success of *A. stolonifera* on Marion Island (Gremmen *et al*., 1998) supports this conclusion. Marion Island's sub-Antarctic location and uniform climate do not provide for an early spring flush of competitors. Rather, the ability to compete on equal terms throughout the year eliminates the early season advantage that many typical competitors enjoy. In the absence of grazing, human interventions and pests of *A. stolonifera*, the colonization of areas of Marion Island appears to be a significant anomaly for this species.

F.4.e. Weed implications of *A. stolonifera* outcrossing

Throughout the more than 75 years of *A. stolonifera* seed production in the Willamette Valley of Oregon (Schoth, 1930), there has been no demonstration that random recombinations within *A. stolonifera* or sexually compatible species have either increased aggressiveness or been detrimental in that environment. Outcrossing is nearly impossible in managed turfgrass settings because normal mowing practices remove reproductive tillers and prevent flowering and pollination (Lush, 1988a). The flowering period for *A. stolonifera* coincides with peak golf play periods of the year that necessitates very frequent clippings, which essentially eliminates any reproductive growth as it appears. *Agrostis stolonifera* in the wild or in general agricultural settings could potentially mature and cross with a compatible individual. However, there is no reason to expect any more aggressive behavior than that observed in the more than 75 years of continuous commercial seed production in the Willamette Valley.

Various works (Jones, 1956b,c; Bradshaw 1958a; Wipff and Fricker, 2001; Belanger, 2003) have demonstrated that interspecific hybridization with *A. stolonifera* is possible. However, the potential for increased risk of weediness has not been demonstrated nor is it a conclusion of most of these studies. Hybrids have also been occasionally observed to be more vegetatively adapted than their parents in habitats intermediate of those preferred by their respective parents (Bradshaw, 1958b). However, the persistence of any hybrid

offspring through sexual reproduction is unlikely due to the overwhelming sterility of F1 hybrids (Jones, 1956b) and even in the rare case of a fertile F1 hybrid, the fitness of the F2 hybrid is highly questionable (Bradshaw, 1958b).

Higher ploidy cytotypes as would be characteristic of infertile hybrids were not observed to be present in hostile environments while lower ploidy cytotypes were fertile and present in all environments (Kik *et al*., 1993). The implication is that there is a survival advantage to sexual reproduction regardless of environment. Since strategic adaptability is a foundation of weed success (Holt, 1988), sterile hybrids are highly unlikely to persist in nature or establish themselves as new weed pests. Insertion of the *cp4 epsps* gene is not expected to alter the persistence of any hybrid offspring.

G. Summary

Agrostis stolonifera is both a native and naturalized species in the U.S., has been grown on golf courses for over 100 years, and has been grown continuously for commercial seed production in the Willamette Valley of Oregon for more than 75 years in the proximity of several other *Agrostis* species. While it is an undesirable species in some situations, *A. stolonifera* is not considered a serious economic pest in any setting except in pedigreed seed fields of other grass species in the U.S. Pacific Northwest. Even in the latter case, it has not been reported as one of the 10 most common weed or crop contaminants of turfgrass seed tested by the Oregon State University Seed Lab (Oregon State University, 2001a). *A. stolonifera* is not unique in this aspect because many grass species are considered weeds when they occur in turfgrass seed production fields. Even genetic off-types of the turfgrass seed species being commercially produced are considered serious and must be removed to qualify for Certified production. Seed producers employ very aggressive, integrated field management programs to maintain the purity of their product and weed management is a critical part of those efforts.

Agrostis stolonifera does not possess many of the characteristics common to serious weeds of agriculture or the environment. While it is a perennial species, it is not a relentless colonizer because of its need for both low stress and high disturbance environments. Consequently, *A. stolonifera* typically establishes in only modest areas within mixed plant communities. Therefore, the impact of *A. stolonifera* contamination within other turfgrass species is primarily an aesthetic rather than a functional or economic issue. Finally, although *A. stolonifera* can cross with related species to form hybrids, they are rarely found, largely sterile, typically intermediate to the parent species and pose essentially no risk to agriculture or the environment.

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III. Description of the Transformation System

A. Characteristics of the recipient plant material

The creeping bentgrass plant tissue that received DNA conferring tolerance to glyphosate was embryogenic plant callus derived from a single seed of the creeping bentgrass cultivar Backspin. This plant and tissue was chosen for insertion of the *cp4 epsps* gene because it responds well to particle bombardment transformation and tissue culture regeneration. B99061R was developed from randomly selected non-transgenic embryogenic callus regenerate in tissue culture and further maitained by vegetative propagation.

B. Description of the transformation system

Biolistic transformation (microprojectile or particle bombardment) was used to produce event ASR368. This DNA delivery system is well documented to transfer and integrate new DNA into a plant genome (Klein *et al.,* 1987; Lee; 1996; Sanford *et al.,* 1993). Prior to bombardment, DNA containing the *cp4 epsps* gene was precipitated onto microscopic gold particles using calcium chloride and spermidine. The precipitated DNA and particles were then placed onto a plastic macrocarrier and accelerated at high velocity such that a stopping screen retained the macrocarrier. The particles with DNA were permitted to continue their flight and eventual penetration and incorporation into the creeping bentgrass plant cells. These cells were transferred to a selective media containing glyphosate and only those cells transformed with the *cp4 epsps* gene continued to grow. Event ASR368 was selected among these transformed plant cells. The flowchart in Figure III.1 illustrates the development of event ASR368.

C. References

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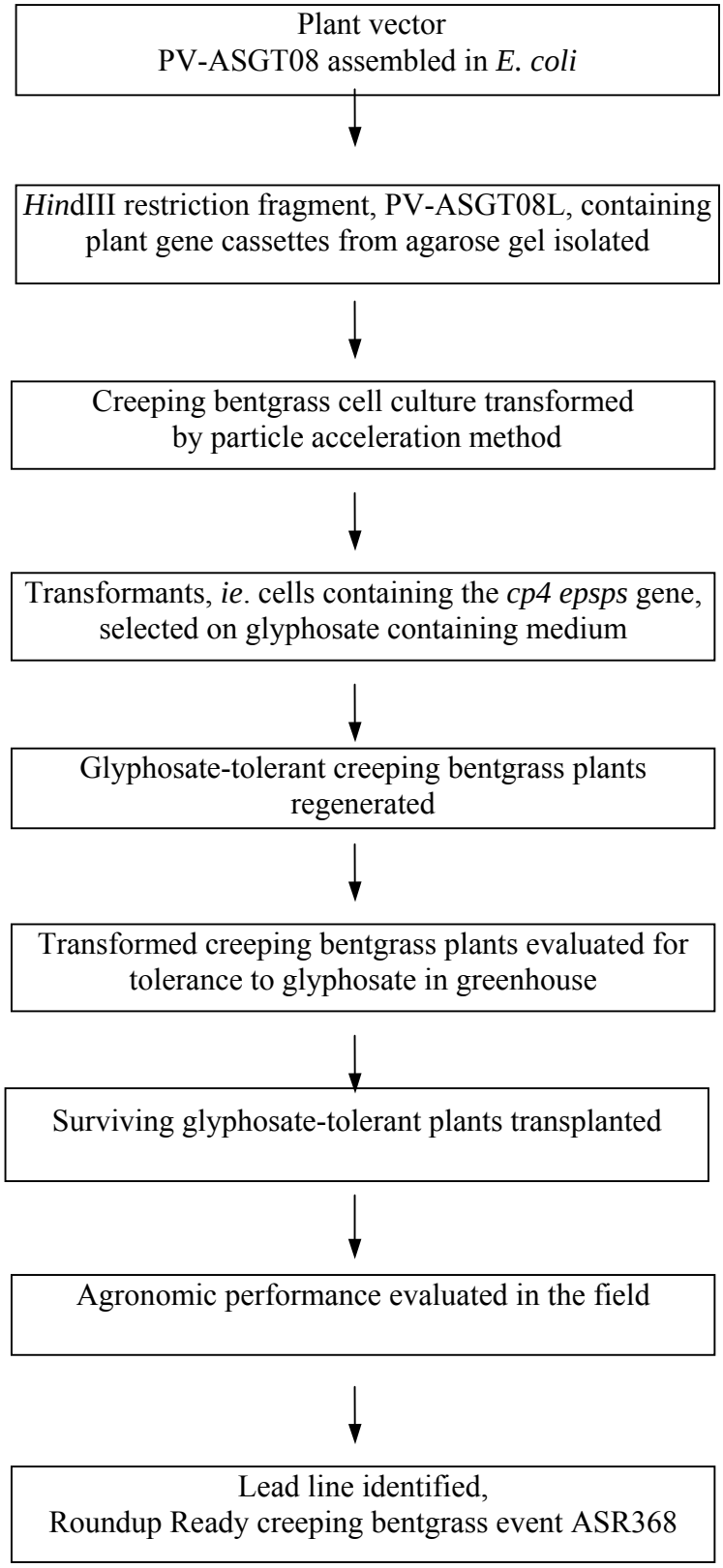
Figure III.1. Development and selection of Roundup Ready creeping bentgrass event ASR368



IV. Donor Genes and Regulatory Sequences

A. Vector PV-ASGT08L

Event ASR368 was produced by transformation of non-transgenic bentgrass tissue with a ~ 6.7 kb linear *Hin*d III DNA derived from the plasmid vector PV-ASGT08 (Figure IV.1) developed by Monsanto Company (St. Louis, MO). This linear segment, PV-ASGT08L (Figure IV.2), contained two *cp4 epsps* gene expression cassettes. The first *cp4 epsps* gene expression cassette contained the *cp4 epsps* coding sequence under the regulation of the rice actin promoter, a rice actin intron, a chloroplast transit peptide (CTP2) sequence and a nopaline synthase (NOS) 3' polyadenylation sequence. The second *cp4 epsps* gene expression cassette contained the *cp4 epsps* coding sequence under the regulation of the cauliflower mosaic virus (CaMV) enhanced 35S plant promoter (e35S), a maize heat-shock protein 70 (*Zm*HSP70) intron, CTP2 and the NOS 3' polyadenylation sequence. The *ctp2 cp4 epsps* coding region used to produce event ASR368 is the same as that employed in several other Roundup Ready crops such as soybean, which have been previously reviewed and granted nonregulated status by the USDA. A description of the elements in the linear DNA segment PV-ASGT08L is provided in Table IV.1.

B. The *cp4 epsps* gene and CP4 EPSPS protein

Event ASR368 plants contain two copies of the *cp4 epsps* gene that encode the CP4 EPSPS protein, which imparts tolerance to glyphosate. The *cp4 epsps* gene was isolated originally from *Agrobacterium* sp. strain CP4 and produces an enzyme, 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) (Padgette *et al.*, 1995). This enzyme, unlike most native plant and microbial EPSPS enzymes, is naturally tolerant to glyphosate (Padgette *et al.*, 1995). EPSPS catalyzes the formation of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase from shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) in both microorganisms and plants. EPSP is an intermediate required for the production of aromatic amino acids (Herrmann, 1983; Haslam, 1974).

The native *Agrobacterium* gene sequence was modified to create a synthetic gene that allows greater production of the CP4 EPSPS protein in plants (Padgette *et al.*, 1995). Bacterial genes, like those from *Agrobacterium,* have several features that reduce their ability to function efficiently in plants. Therefore, plant-preferred versions of these genes were synthesized and used in developing the plasmid vectors (Della-Cioppa *et al.*, 1986 and 1987; Shah *et al.*, 1986).

The *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 has been completely sequenced and encodes a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids (Padgette *et al.*, 1996). The *ctp*2 *cp4 epsps* gene sequence, present in event ASR368, is approximately 1.7 kb in size. The deduced amino acid sequence of the CP4 EPSPS protein with the CTP2 transit peptide is shown in Figure IV.3.

The target for glyphosate in plants, the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme, is located in the chloroplast. Many chloroplast-localized proteins, including EPSPS, are expressed from nuclear genes as precursors and are targeted to the chloroplast by a chloroplast transit peptide (CTP) that is removed during the import process. It has been demonstrated *in vivo* (Timko *et al.*, 1988) and *in vitro* (Della-Cioppa *et al.*, 1986 and 1987) that non-chloroplast proteins may be targeted to the chloroplast by use of protein hybrids containing a CTP and that a CTP amino acid sequence is sufficient to target a protein to the chloroplast. The *ctp* coding sequence from the *Arabidopsis thaliana epsps* coding region (Klee *et al.*, 1987) was joined to the *cp4 epsps* coding sequence to achieve chloroplast localization of CP4 EPSPS protein. The CP4 EPSPS protein, as a bacterial protein, contains no CTP. The *Arabidopsis ctp* DNA sequence was modified by site-directed mutagenesis to place a *Sph* I restriction site at the CTP processing site to accomplish this joining. This change replaced the Glu-Lys at this location with Cys-Met. The DNA sequence of this CTP peptide is designated as *ctp2*. The CTP2 CP4 EPSPS hybrid protein was demonstrated to allow import into chloroplasts isolated from *Lactuca sativa*, using methods described previously (Della-Cioppa *et al.*, 1986 and 1987).

C. The chloroplast transit peptide (CTP2)

In both plant gene expression cassettes, the *cp4 epsps* coding sequence is fused to a chloroplast transit peptide (designated CTP2) whose sequence is based on the CTP isolated from *Arabidopsis thaliana* EPSPS (Klee *et al.*, 1987). This CTP directs the CP4 EPSPS protein to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid biosynthesis (Kishore and Shah, 1988). CTPs are typically cleaved from the "mature" protein following delivery to the plastid (della-Cioppa *et al.*, 1986).

D. Regulatory sequences

In the first gene cassette, the *cp4 epsps* coding sequence is under the control of the 5' non-coding end of the rice actin 1 sequence (ract1) containing the promoter and first intron (McElroy *et al*., 1990), approximately 1.4 kb in size, introduced upstream of the CTP sequence. The second cassette contains the *cp4 epsps* coding sequence under the control of the enhanced CaMV 35S promoter (e35S) (Kay *et al*., 1987; Odell *et al*., 1985), which is approximately 0.6 kb in size. Located between the e35S promoter and the *cp4 epsps* sequence is the 0.8-kb intron from the corn *hsp*70 (heat shock protein), present to increase the levels of gene transcription (Rochester *et al*., 1986). In each cassette, the *cp4 epsps* sequence is joined to the 0.3-kb nopaline synthase 3' nontranslated sequence, NOS 3', from *Agrobacterium tumefaciens* (Fraley *et al*., 1983), which provides the transcription termination and the mRNA polyadenylation signal.

An origin of replication sequence (*ori*) is also present in PV-ASGT08 to allow for the replication of the plasmid in *E. coli* (Viera and Messing, 1987). Following the *ori* region is the DNA sequence encoding the enzyme *neomycin phosphotransferase type II* (*nptII*). This enzyme confers resistance to aminoglycoside antibiotics (e.g., kanamycin and

neomycin) and was used for selection of bacteria during the construction of the plasmid. The coding sequence for the *nptII* gene was derived from the prokaryotic transposon Tn5 (Beck *et al*., 1982) and is present with its own bacterial promoter. The fragment, used for the transformation of event ASR368, contains neither the *ori* or the *nptII* sequences.

The plasmid PV-ASGT08 was amplified in *E. coli* and purified from bacterial lysates. The *cp4 epsps* gene expression linear DNA fragment was isolated from the plasmid prior to creeping bentgrass transformation experiments by digesting PV-ASGT08 with the restriction enzyme *Hind* III. The plasmid backbone (~2.6 kb) and the *cp4 epsps* expression cassettes (~6.7 kb) were separated by gel electrophoresis and the *cp4 epsps* expression cassette fragment was electro-eluted from a gel slice. The agarose gel-isolated *Hind* III restriction fragment utilized in the transformation of Roundup Ready creeping bentgrass event ASR368 was designated PV-ASGT08L. This fragment contains neither the *ori* or *nptii* sequences.

E. References

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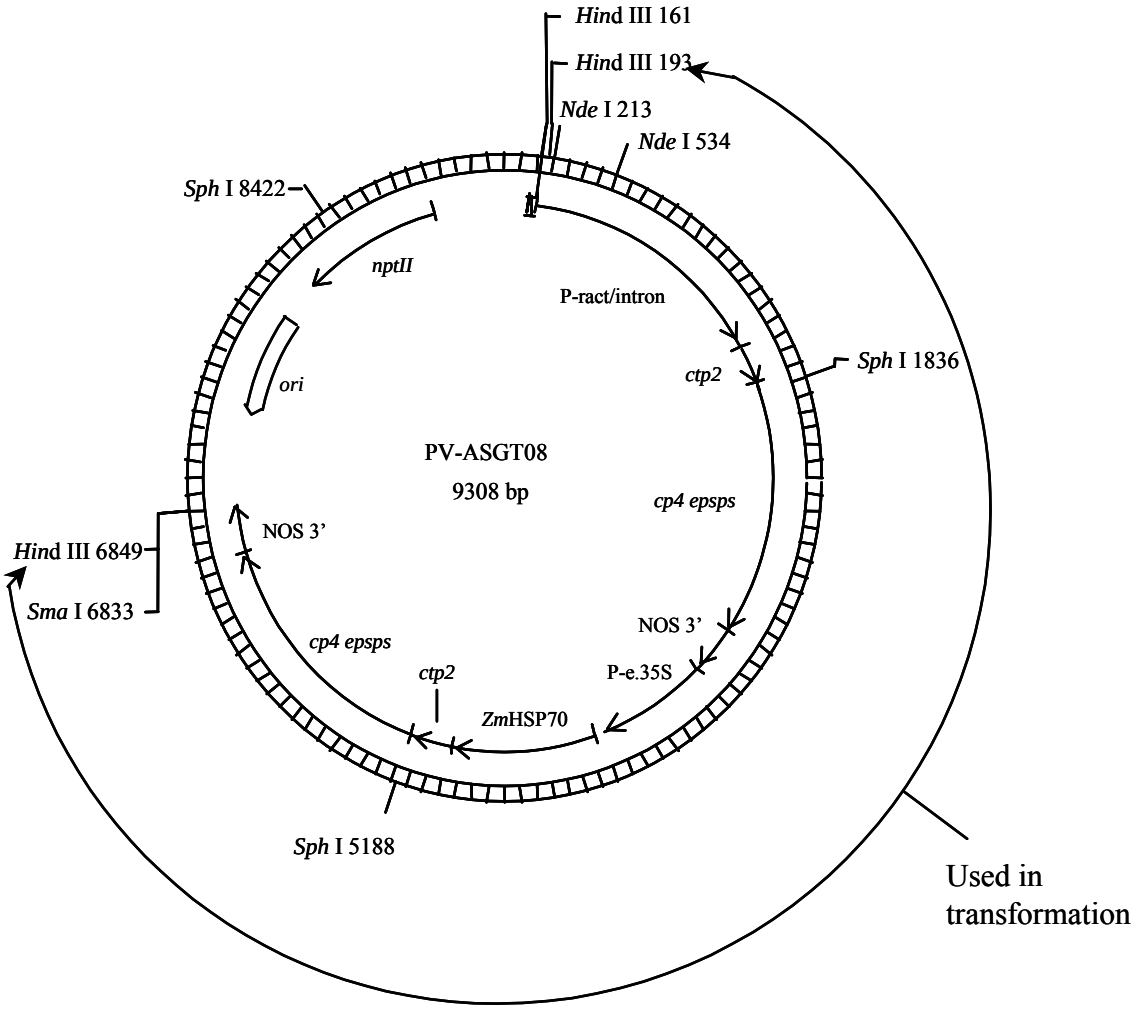
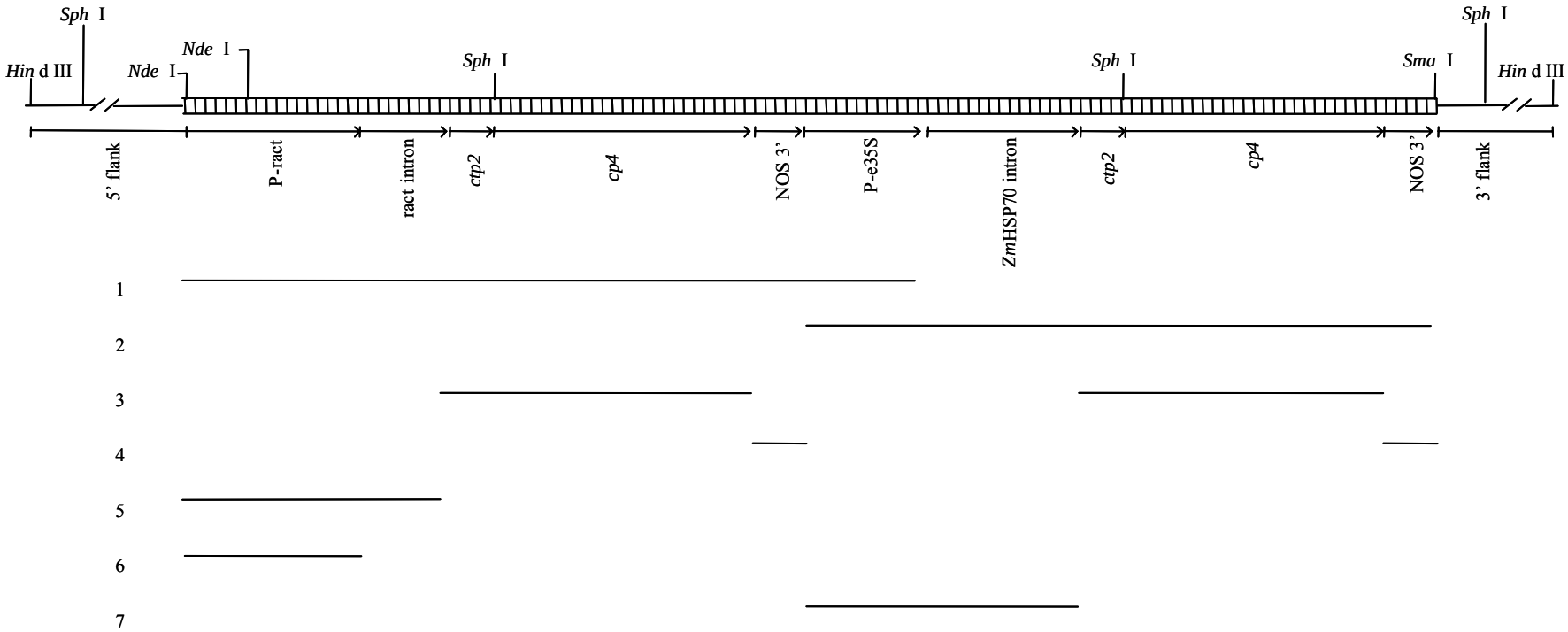
Figure IV.1. Plasmid Map of PV-ASGT08

Circular map of the plasmid vector PV-ASGT08 is shown with genetic elements annotated. Restriction sites with positions relative to the size of the plasmid vector for enzymes used in the Southern analysis are shown. Probes used in the Southern analysis are detailed in the accompanying table.





Probe Number	Probe Name	Start Position	End Position	Total Length (bp)
1	Insert Probe #1	180	4062	3883
2	Insert Probe #2	3446	6874	3409
3	*ctp2 -cp4 epsps*	1608, 4958	3167, 6517	1560
4	NOS 3'	3217, 6567	3472, 6822	256
5	P-ract + ract1 intron	266	1582	1317
6	P-ract	175	1150	976
7	P-e35S+ *Zm* HSP70 intron	3474	4936	1463

Figure IV.2. Map of the insert in Roundup Ready creeping bentgrass event ASR368.
A schematic of the predicted DNA insert in Roundup Ready creeping bentgrass event ASR368 based on Southern blot analysis, PCR and nucleotide sequencing.

Figure IV.3. Deduced amino acid sequence of the CP4 EPSPS protein
Sequence includes the CTP2 transit peptide (amino acids 1-76 are the transit peptide).

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  1  MAQVSRICNG VQNPSLISNL SKSSQRKSPL SVSLKTQQHP RAYPISSSWG
 51  LKKSGMTLIG SELRPLKVMS SVSTACMLHG ASSRPATARK SSGLSGTVRI
101  PGDKSISHRS FMFGGLASGE TRITGLLEGE DVINTGKAMQ AMGARIRKEG
151  DTWIIDGVGN GGLLAPEAPL DFGNAATGCR LTMGLVGVYD FDSTFIGDAS
201  LTKRPMGRVL NPLREMGVQV KSEDGDRLPV TLRGPKTPTP ITYRVPMASA
251  QVKSAVLLAG LNTPGITTVI EPIMTRDHTE KMLQGFGANL TVETDADGVR
301  TIRLEGRGKL TGQVIDVPGD PSSTAFPLVA ALLVPGSDVT ILNVLMNPTR
351  TGLILTLQEM GADIEVINPR LAGGEDVADL RVRSSTLKGV TVPEDRAPSM
401  IDEYPILAVA AAFAEGATVM NGLEELRVKE SDRLSAVANG LKLNGVDCDE
451  GETSLVVRGR PDGKGLGNAS GAAVATHLDH RIAMSFLVMG LVSENPVTVD
501  DATMIATSFP EFMDLMAGLG AKIELSDTKA A
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Table IV.1. Summary of the genetic elements in plasmid PV-ASGT08

Genetic Element	Position in PV-ASGT08	Function
P-ract1/ract1 intron	199-1592	The 5' region of rice (*Oryzae sativa*) actin1gene containing the promoter, transcription start site and first intron. (McElroy *et al*., 1990)
ctp2	1609-1836	The DNA sequence for chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS; transit peptide directs the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid biosynthesis.
cp4 epsps	1837-3204	The coding sequence for the native 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium sp*. strain CP4.
NOS 3'	3217-3472	The 3' nontranslated region of the nopaline synthase (NOS) coding sequence from *Agrobacterium tumifaciens* which terminates transcription and directs polyadenylation (Fraley *et al*., 1983).
P-e35S	3489-4101	The cauliflower mosaic virus (CaMV) promoter (Odell *et al*., 1985) with the duplicated enhancer region (Kay *et al*., 1987) used to drive expression of the *ctp2-cp4 epsps* coding region.
Zm HSP70	4131-4934	The intron from the maize (*Zea mays*) *hsp70* gene (heat shock protein) present to stabilize the level of gene transcription.
ctp2	4959-5186	The DNA sequence for chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS; transit peptide directs the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid biosynthesis.

Table IV.1. Summary of the genetic elements in plasmid PV-ASGT08 (continued).

Genetic Element	Position in PV-ASGT08	Function
cp4 epsps	5187-6554	The coding sequence for the native 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium sp*. Strain CP4.
NOS 3'	6567-6822	The 3' nontranslated region of the nopaline synthase (NOS) coding sequence from *Agrobacterium tumifaciens,* which terminates transcription and directs polyadenylation (Fraley *et al*., 1983).
ORI	7251-7905	The minimum pBR322 sequence required for plasmid replication. Sequence downstream of this region is known to affect copy number.
NPTII	7986-8969	The gene for the enzyme neomycin phosphotransferase type II from Tn5, a transposon isolated from *Escherichia coli (*Beck *et al.,* 1982). The *npt*II gene also contains a 0.153 kb portion of the 0.378 kb *ble* gene from Tn5.

V. Genetic Analysis

A. Molecular characterization of Roundup Ready creeping bentgrass event ASR368

Southern blot, PCR and nucleotide sequence analyses were performed to characterize the DNA integrated into the genome of event ASR368. These analyses support the following conclusions: (1) the genome of event ASR368 contains a single DNA insertion comprised of a single copy of the DNA segment used for transformation; (2) both *cp4 epsps* gene expression cassettes within the single insert are intact; (3) transcription of the first *cp4 epsps* gene expression cassette contains the *cp4 epsps* coding sequence under the regulation of the rice actin promoter, a rice actin intron, a chloroplast transit peptide (CTP2) sequence from *A. thaliana* and a nopaline synthase (NOS) 3' polyadenylation sequence from *A. tumefaciens* and the second *cp4 epsps* gene expression cassette contains the *cp4 epsps* coding sequence under the regulation of the cauliflower mosaic virus (CaMV) enhanced 35S plant promoter (e35S), a maize heat-shock protein 70 (*Zm*HSP70) intron, CTP2 and the NOS 3' polyadenylation sequence; and (4) the genome of event ASR368 does not contain any detectable plasmid backbone DNA.

The 5' and 3' ends of the event ASR368 insert and the *cp4 epsps* coding regions were verified by PCR and DNA sequencing. These data support the conclusion that only the full-length CTP2-CP4 EPSPS protein should be encoded by the insert in event ASR368. The genetic stability of the transgene was also demonstrated by Southern blot analysis on genomic DNA from the R0, F1 and F2 generations of event ASR368.

A.1. Materials and methods

A.1.a. Test substance

The test substance for the study was Roundup Ready creeping bentgrass event ASR368. In addition, leaf material from event ASR368 F1 and F2 generations was used to analyze the stability of the inserted DNA across generations.

A.1.b. Control substances

The control substance for the molecular characterization of Roundup Ready creeping bentgrass events ASR368 was the non-transformed bentgrass line 99061R/990028 (B99061R). In addition, leaf material from the bentgrass line V13-2-2, the maternal parental line of the F1 and F2 generations, was obtained from The Scotts Company for use as a control in analyzing the stability of the inserted DNA across generations.

A.1.c. Reference substances

Plasmid PV-ASGT08, the source plasmid, served as the primary reference substance in these analyses. The plasmid, mixed with DNA from the 99061R/990028 non-transformed

control substance, was used as a size indicator and a positive hybridization control in Southern blot analysis. Additionally, molecular size markers from Gibco BRL [1 Kb DNA extension ladder (40 Kb-0.4 Kb) catalog #10511] were used for size estimations.

A.1.d. Southern blot strategy

Genomic DNA from event ASR368 was digested with restriction enzymes and subjected to Southern blot hybridization analysis to characterize the DNA that was integrated into the bentgrass genome. A map showing the linear DNA segment, PV-ASGT08L, that was used to generate the event ASR-368, along with the locations of the restriction sites utilized for Southern analysis, is shown in Figure IV.2.

DNA from the test substance was digested with *Hin*d III or *Nde* I/*Sma* I on each Southern blot to characterize event ASR368. *Hin*d III does not cleave within the PV-ASGT08L segment and, therefore, gives an indication of the number of insertions in event ASR368. *Nde* I and *Sma* I cleave at the 5' and 3' ends of the PV-ASGT08L segment, respectively. This enzyme combination gives an indication of the number of copies of the PV-ASGT08L segment in event ASR368. The non-transgenic and plasmid DNAs were also digested with *Nde* I/*Sma* I for all blots. Only the P-ract and P-ract/intron blots contained an additional *Hin*d III non-transgenic control digest in the long and short runs, as specified in the protocol. These Southern blots were probed with all of the elements of PV-ASGT08 and with two overlapping probes that collectively span the entire PV-ASGT08L sequence.

The genetic stability of the event ASR368 insert over multiple generations was assessed by digesting genomic DNA from the test substances (R0, F1 and F2) with *Sph* I. This enzyme generates a unique fingerprint for event ASR368 when probed with the *ctp2 cp4 epsps* coding region.

A.1.e. DNA isolation

DNA extracted from leaf tissue was used for all of the analyses in this report. Leaf tissue was frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. Approximately 2 g of the processed leaf was transferred to a 50 ml conical tube and ~16 ml of CTAB extraction buffer [1.5% (w/v) CTAB, 75 mM Tris pH 8.0, 100 mM EDTA pH 8.0, 1.05 M NaCl and 0.75% (w/v) PVP (MW 40,000)] were added to the processed leaf. The samples were incubated at 65°C for approximately 30 minutes with intermittent mixing and then allowed to cool to room temperature. An equal volume (~15 ml) of room temperature chloroform:isoamyl alcohol [24:1 (v/v)] was added to the samples. The suspension was mixed for 5 minutes and the two phases separated by centrifugation at ~16,000 x *g* for 5 minutes at room temperature. The aqueous (top) layer was removed and placed into a clean 50 ml conical tube. Approximately 1/10 volume (~1.5 ml) of 10% CTAB buffer [10% (w/v) CTAB and 0.7 M NaCl] and an equal volume of chloroform:isoamyl alcohol [24:1 (v/v)] were added to the aqueous phase, which was then mixed by inversion for 5 minutes. The samples were centrifuged at ~16,000 x *g* for

5 minutes at room temperature to separate the phases. The aqueous (upper) phase was removed, mixed with an equal volume (~15 ml) of CTAB precipitation buffer [1% (w/v) CTAB, 50 mM Tris pH 8.0 and 10 mM EDTA pH 8.0] and allowed to stand at room temperature for approximately 1 hour. The samples were centrifuged at ~10,000 x *g* to pellet the DNA, the supernatant was decanted and the pellet was dissolved in approximately 2 ml of high salt TE [10mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0 and 1 M NaCl] by incubating at 37°C with gentle swirling for approximately 2 hours or by sitting in a 4°C refrigerator overnight. Centrifugation was performed at ~23,000 x *g* to pellet any remaining impurities. The supernatant was removed, placed into a clean 15 ml tube and approximately 1/10 volume (~150 µl) of 3M NaOAc, pH 5.2 and 2 volumes (~4 ml relative to the supernatant) of 100% ethanol were added to precipitate the DNA. The precipitated DNA was spooled into a microfuge tube containing 70% ethanol. The DNA was pelleted in a microfuge at maximum speed (14,000 rpm) for ~ 5 minutes, dried and re-dissolved in TE, pH 8.0 in a 4°C refrigerator overnight.

A.1.f. DNA quantitation

The purified genomic DNA was quantitated using a Hoefer DyNA Quant™ 200 Fluorometer (San Francisco, CA) with Roche Molecular Weight Marker IX (catalog #1449 460) used as a calibration standard.

A.1.g. Restriction enzyme digestion

Approximately 10 µg of genomic DNA from the test event and control lines were used for the restriction enzyme digests. Overnight digests were performed at 37°C in a total volume of 500 µl using 100 units of each restriction enzyme. Some of the control digests were spiked with either 10 or 20 pg of plasmid PV-ASGT08 instead of ½ and 1 copy number equivalents due to limited information about the genome size of *Agrostis stolonifera*. After digestion, the samples were precipitated by adding 1/10 volume (~50 µl) of 3M NaOAc and 2 volumes (~1 ml relative to the original digest volume) of 100% ethanol, followed by incubation at -20°C for at least one hour. The digested DNA was pelleted by centrifugation, washed with 70% ethanol, vacuum dried for approximately 4 minutes in a vacufuge and re-dissolved at room temperature in water.

A.1.h. Agarose gel electrophoresis

Digested DNAs were separated on 0.8% agarose gels in 1X TBE buffer. A 'long run' and a 'short run' were performed for each Southern blot analysis, except for the generational stability blot that only contained a single run. The long run facilitated greater resolution of the higher molecular weight DNAs while the short run ensured that all smaller molecular weight DNAs were retained on the gel. The long run/short run involved a 4-6 hour electrophoresis at 80-85 V and an overnight (15.5-19 hours) run at 35-38 V. After electrophoresis, the gels were stained in 0.5 µg/ml ethidium bromide for 20-30 minutes and photographed.

A.1.i. DNA probe preparation

Plasmid PV-ASGT08 DNA was isolated from *E. coli* cultures. DNA probe templates homologous to the P-ract, plasmid backbone, NOS 3' polyadenylation sequence, insert probe #1 (containing the P-ract1/ract1 intron, *ctp2-cp4 epsps*, NOS 3' polyadenylation sequence, and the e35S promoter), and insert probe #2 (containing the e35S promoter, *Zm*HSP70 intron, *ctp2-cp4 epsps*, the NOS 3' polyadenylation sequence) were prepared by PCR using plasmid PV-ASGT08 as a template. A single plasmid backbone probe was prepared instead of two to three overlapping probes as specified in the protocol. The probe templates for the P-ract1/ract1 intron, e35S promoter/*Zm*HSP70 intron and *ctp2-cp4 epsps* were prepared by PCR using gel purified restriction fragments from PV-ASGT08.

Approximately 25 ng of each probe template was labeled with ^{32}P-CTP using the random priming method (RadPrime DNA Labeling System, Gibco BRL). The radiolabeled probe was purified prior to hybridization using a Sephadex G-50 column (Roche).

A.1.j. Southern blot analyses

Southern blot analyses (Southern, 1975) were performed according to SOP GEN-PRO-025-02. Following electrophoresis, the gel was placed in depurination solution (0.125 N HCl) for ~10 minutes followed by denaturing solution (0.5 M NaOH, 1.5 M NaCl) for ~30 minutes, and then neutralizing solution (0.5 M Tris-HCl pH 7, 1.5 M NaCl) for ~30 minutes. The DNA from the agarose gels was transferred to Hybond-N nylon membranes (Amersham) using a Turboblotter™ (Schleicher & Schuell). The DNA was allowed to transfer for 4 hours to overnight (in 20X SSC) and covalently cross-linked to the membrane with a UV Stratalinker™ 1800 (Stratagene) set to autocrosslink. The blots were prehybridized for approximately 45 minutes-5 hours in an aqueous solution of 0.5 M sodium phosphate, 7% SDS (w/v) and 0.1 mg/ml *E. coli* tRNA. Hybridization with the radiolabeled probe was performed in fresh prehybridization solution for 15.5-22.5 hours at approximately 65°C. Membranes were washed in an aqueous solution of 0.1% (w/v) SDS and 0.1X SSC for two 15 minute intervals, followed by two 20 minute intervals at 65°C. Multiple exposures of the blots were generated using Kodak Biomax MS film in conjunction with one Kodak Biomax MS intensifying screen.

A.1.k. Verification of genomic DNA sequences flanking the 5' and 3' ends of the insert

The sequences of the 5' and 3' insert to plant genomic DNA junctions were determined using Clontech's Universal Genome Walker™ Kit and the RAGE method (Rapid Amplification of Genomic DNA Ends) and verified by PCR. The 5' junction was verified using one primer designed to the genomic DNA sequence flanking the 5' end of the insert paired with a second primer in the rice actin promoter of the inserted DNA. The 5' junction was verified using 50 ng of leaf genomic DNA (1 µl) as a template, 15 pmol of each primer (1.5 µl each) and the Expand High Fidelity PCR system (Roche) in

a 50 µl reaction volume. The amplification of the reactions was performed under the following cycling conditions: 1 cycle at 94°C for 2 minutes; 10 cycles at 94°C for 15 seconds, 60°C for 30 seconds, 72°C for 1 minute; 25 cycles at 94°C for 15 seconds, 60°C for 30 seconds, 72°C for 1 minute + 5 additional seconds per cycle; 1 cycle 72°C for 7 minutes.

The 3' junction was verified using one primer designed to the genomic DNA sequence flanking the 3' end of the insert paired with a second primer located in the NOS 3' polyadenylation sequence of the inserted DNA. The PCRs were conducted using 211 ng of leaf genomic DNA (1 µl) as a template, 15 pmol of each primer (1.5 µl each), and the Expand Long Template PCR system (Roche) in a 50 µl reaction volume. The amplification of the reactions was performed under the following cycling conditions: 1 cycle at 94°C for 2 minutes; 35 cycles at 94°C for 10 seconds, 60°C for 30 seconds, 68°C for 30 seconds; 1 cycle at 68°C for 10 minutes.

All PCR products were separated on a 1% TAE agarose gel and visualized by staining with ethidium bromide. Separated PCR products were purified from the agarose matrix and subjected to DNA sequencing using dye-terminator chemistry (Monsanto Genomics Sequencing Center) to confirm the sequences.

A.1.l. PCR analysis and sequence confirmation of the *cp4 epsps* coding regions

The linkage of the elements contained within the event ASR368 insert was confirmed by generating two overlapping PCR products that spanned the length of the insert. The PCR analyses were conducted multiple times using 192 ng of genomic DNA as a template in a 50 µl reaction volume containing a final concentration of 1.75 mM $MgCl_2$, 0.3 µM of each primer, 350 µM each dNTP and 2.5 units of Expand Long Template enzyme mix (Roche). The reactions for the 5' end of the insert were performed under the following cycling conditions: 94°C for 2 minutes; 10 cycles at 94°C for 10 seconds, 65°C, decreasing 1°C per cycle, for 30 seconds, 68°C for 4 minutes; 25 cycles at 94°C for 10 seconds, 56°C for 30 seconds, 68°C for 4 minutes increasing 20 seconds per cycle; 1 cycle at 68°C for 10 minutes. The reactions for the 3' end of the insert were performed under the following cycling conditions: 94°C for 2 minutes; 10 cycles at 94°C for 10 seconds, 65°C, decreasing 1°C per cycle, for 30 seconds, 68°C for 2 minutes; 25 cycles at 94°C for 10 seconds, 56°C for 30 seconds, 68°C for 2 minutes increasing 20 seconds per cycle; 1 cycle at 68°C for 10 minutes. The PCR products were either separated using 1.0 % agarose gel electrophoresis according to SOP # GEN-PRO-003-01 or purified directly from the PCR reaction mixture using the QIAquick PCR Purification Kit (Qiagen). The PCR products derived from event ASR368 that were separated using agarose gel electrophoresis were excised from the gel and purified using the QIAquick Gel Extraction Kit (Qiagen) following the procedure supplied by the manufacturer. The purified PCR products were then sequenced with the initial PCR primers as well as primers designed internal to the amplified sequences. All sequencing was performed by the Monsanto Genomics Sequencing Center using dye-terminator chemistry. A

consensus sequence was created from interpretable sequence runs using DNAstar/SeqManII version 5.00.

A.2. Results and discussion

A.2.a. Insert and copy number

A.2.a.1. Insert probe #1

A probe containing the first *cp4 epsps* expression cassette and the e35S promoter (Figure IV.1) was used to probe event ASR368. The results are shown in Figure V.1. The 99061R/990028 control *Nde* I/*Sma* I long run (lane 1) showed a low level of background hybridization. Plasmid PV-ASGT08 mixed with the 99061R/990028 control *Nde* I/*Sma* I short run digests (lanes 4 and 5) produced the expected size band at approximately 6.3 kb, in addition to the low background hybridization. The event ASR368 *Hin*d III long and short run digests (lanes 2 and 6) each produced one band at approximately 10 Kb, in addition to the low level background hybridization (also seen in Figures V.4 and V.5, lanes 1 and 7). The event ASR368 *Nde* I/*Sma* I long and short run digests (lanes 3 and 7) each produced a single band not present in the 99061R/990028 control lanes at approximately 6.3 Kb. The presence of a single band in each lane suggests that event ASR368 contains a single, intact copy of integrated DNA located on a 10 Kb *Hin*d III restriction segment.

The predicted 321 bp band from the *Nde* I to *Nde* I sites, corresponding to the 5' portion of the rice actin promoter, is not detected on this blot. This is presumably due to the low percentage of the total probe template that this portion represents.

A.2.a.2 Insert probe #2

A probe containing the second *cp4 epsps* expression cassette (Figure IV.1) was used to probe event ASR368. The results are shown in Figure V.2. The 99061R/990028 control *Nde* I/*Sma* I long run digest (lane 1) did not produce any detectable background bands. Plasmid PV-ASGT08 mixed with the 99061R/990028 control *Nde* I/*Sma* I short run digests (lanes 4 and 5) produced the expected size band at approximately 6.3 kb with no detectable background bands. The event ASR368 *Hin*d III long and short run digests (lanes 2 and 6) each produced one band at approximately 10 kb. The event ASR368 *Nde* I/*Sma* I long and short run digests (lanes 3 and 7) each produced one band at approximately 6.3 kb. These results also suggest that event ASR368 contains a single, intact copy of integrated DNA located on a 10 kb *Hin*d III restriction segment.

A.2.b. *ctp2-cp4 epsps* coding region intactness

A probe containing the *ctp2-cp4 epsps* coding region (Figure IV.1) was used to probe event ASR368. The results are shown in Figure V.3. The 99061R/990028 control *Nde* I/*Sma* I long run digest (lane 1) did not produce any detectable background bands.

Plasmid PV-ASGT08 mixed with the 99061R/990028 control *Nde* I/*Sma* I short run digests (lanes 4 and 5) produced the expected size band at approximately 6.3 kb with no detectable background bands. The event ASR368 *Hin*d III long and short run digests (lanes 2 and 6) each produced one band at approximately 10 kb. The event ASR368 *Nde* I/*Sma* I long and short run digests (lanes 3 and 7) each produced one band at approximately 6.3 kb. These results suggest that event ASR368 contains a single segment of integrated DNA located on a 10 kb *Hin*d III restriction segment; no additional detectable DNA segments were detected using this probe.

A.2.c. Rice actin promoter/intron probe

A probe containing the rice actin promoter/intron (Figure IV.1) was used to probe event ASR368. The results are shown in Figure V.4. The 99061R/990028 control long run digests [lanes 1 (*Hin*d III) and 2 (*Nde* I/*Sma* I)] showed a high level of background hybridization. Plasmid PV-ASGT08 mixed with the 99061R/990028 control *Nde* I/*Sma* I short run digests (lanes 5 and 6) produced the expected size bands at approximately 6.3 kb and 0.32 kb (very faint), in addition to the high background hybridization. The 99061R/990028 control *Hin*d III short run digest (lane 7) also showed a high level of background hybridization. The event ASR368 *Hin*d III long and short run digests (lanes 3 and 8) each produced one band not present in their respective 99061R/990028 control lanes at approximately 10 kb. The event ASR368 *Nde* I/*Sma* I long and short run digests (lanes 4 and 9, respectively) each produced one band not present in their respective 99061R/990028 control lanes at approximately 6.3 kb. The expected, faint, 0.32 kb band can be seen in the event ASR368 *Nde* I/*Sma* I short run sample. These results confirm data suggesting that event ASR368 contains a single, intact segment of integrated DNA located on a 10 kb *Hin*d III restriction segment and also establish that event ASR368 contains the intact rice actin promoter/intron with no additional detectable DNA segments detected using this probe.

A.2.d. Rice actin promoter probe

High levels of background hybridization were observed when probing with the rice actin promoter/intron. Similar high levels of background hybridization have been reported to the USDA in other monocots using the same probe (USDA Petition 00-011-01p). Due to the high level of background hybridization found (Figure V.4) a similar blot was probed with the rice actin promoter alone. The results are shown in Figure V.5. The 99061R/990028 control long run digests [lanes 1 (*Hin*d III) and 2 (*Nde* I/*Sma* I)] showed a moderate level of background hybridization. Plasmid PV-ASGT08 mixed with the 99061R/990028 control *Nde* I/*Sma* I short run digests (lanes 5 and 6) produced the expected size bands at approximately 6.3 kb and 0.32 kb (very faint), in addition to the moderate background hybridization. The 99061R/990028 control *Hin*d III short run digest (lane 7) also showed a moderate level of background hybridization. The event ASR368 *Hin*d III long and short run digests (lanes 3 and 8) each produced one band not present in their respective 99061R/990028 control lanes at approximately 10 kb. The event ASR368 *Nde* I/*Sma* I long and short run digests (lanes 4 and 9) each produced one

band not present in their respective 99061R/990028 control lanes at approximately 6.3 kb. The expected 0.32 kb band can be seen hybridizing very faintly in the event ASR368 *Nde* I/*Sma* I short run sample. These results confirm data suggesting that event ASR368 contains a single, intact segment of integrated DNA located on a 10 kb *Hin*d III restriction segment and also establish that event ASR368 contains the intact rice actin promoter with no additional detectable DNA segments detected using this probe.

A.2.e. NOS 3' polyadenylation sequence probe

A probe containing the NOS 3' polyadenylation sequence (Figure IV.1) was used to probe event ASR368. The results are shown in Figure V.6. The 99061R/990028 control *Nde* I/*Sma* I long run digest (lane 1) did not produce any detectable background bands. Plasmid PV-ASGT08 mixed with the 99061R/990028 control *Nde* I/*Sma* I short run digests (lanes 4 and 5) produced the expected size band at approximately 6.3 kb with no detectable background bands. The event ASR368 *Hin*d III long and short run digests (lanes 2 and 6) each produced one band at approximately 10 kb. The event ASR368 *Nde* I/*Sma* I long and short run digests (lanes 3 and 7) each produced one band at approximately 6.3 kb. These results confirm data suggesting that event ASR368 contains a single segment of integrated DNA located on a 10 kb *Hin*d III restriction segment and also establish that event ASR368 contains the intact NOS 3' polyadenylation sequences, with no additional detectable DNA segments detected using this probe.

A.2.f. Enhanced 35S promoter/*Zm*HSP70 intron probe

A probe containing the enhanced 35S promoter and *Zm*HSP70 intron (Figure IV.1) was used to probe event ASR368. The results are shown in Figure V.7. The 99061R/990028 control *Nde* I/*Sma* I long run digest (lane 1) did not produce any detectable background bands. Plasmid PV-ASGT08 mixed with the 99061R/990028 control *Nde* I/*Sma* I short run digests (lanes 4 and 5) produced the expected size band at approximately 6.3 kb with no detectable background bands. The event ASR368 *Hin*d III long and short run digests (lanes 2 and 6) each produced one band at approximately 10 kb. The event ASR368 *Nde* I/*Sma* I long and short run digests (lanes 3 and 7) each produced one band at approximately 6.3 kb. These results confirm data suggesting that event ASR368 contains a single segment of integrated DNA located on a 10 kb *Hin*d III restriction segment and also establish that event ASR368 contains the intact enhanced 35S promoter and *Zm*HSP70 intron sequences, with no additional detectable DNA segments detected using this probe.

A.2.g. Analysis for backbone fragments

A probe containing the PV-ASGT08 backbone sequence (Figure IV.1) was used to analyze event ASR368. The results are shown in Figure V.8. The 99061R/990028 control *Nde* I/*Sma* I long run digest (lane 1) did not produce any detectable background bands. Plasmid PV-ASGT08 mixed with the 99061R/990028 control *Nde* I/*Sma* I short run digests (lanes 4 and 5) produced a band that appears to migrate slightly above 3.1 kb

with no detectable background bands. The apparent shift in migration of the plasmid can be attributed to the method used to record the molecular weight markers on the autoradiograph. The molecular weight markers were cut from the membrane prior to hybridization. After exposure of the membrane to film, the removed markers were realigned with the membrane and the sizes of the markers were indicated on the films. A small error occurred when the markers were realigned with the membrane. The event ASR368 *Hin*d III long and short run digests (lanes 2 and 6) showed no hybridization. The event ASR368 *Nde* I/*Sma* I long and short run digests (lanes 3 and 7) showed no hybridization. This result establishes that event ASR368 does not contain any detectable plasmid backbone sequence as none was detected using this probe.

A.2.h. Genetic stability

Genomic DNA from the test and control substances was digested with *Sph* I and probed with the *ctp2-cp4 epsps* coding region. The results are shown in Figure V.9. The V13-2-2 and 99061R/990028 control lanes did not produce any detectable background bands (lanes 4 and 5, respectively). Plasmid PV-ASGT08 mixed with the 99061R/990028 control digest (lane 6) produced the expected size bands at approximately 3.35, 3.23 (closely migrated) and 2.72 kb with no detectable background bands. The R0, F1 and F2 test substance lanes (lanes 1-3, respectively) produced three bands at 8.3, 6.2, and 3.35 kb. The 3.35 kb band represents the internal *Sph* I sequence, while the 8.3 and 6.2 kb bands represent border segments. The 8.3 kb band is a result of the border segment at the 3' end of the insert. The less intense, 6.2 kb band is a result of the border segment at the 5' end of the insert. This 6.2 kb band is less intense than the others because a smaller portion of the target sequence is located on this segment. This result establishes the stability of the insert in event ASR368 over multiple plant breeding generations.

A.2.i. Genomic sequence flanking the insert

PCR was performed on genomic DNA to confirm the insert to plant junction sequences at the 5' and 3' ends of the event ASR368 insert. The results of these PCRs are shown in Figure V.10. Panel A shows the result of the PCR confirmation of the 5' insert to plant junction. As expected, the non-transformed control and the no template control (lanes 4 and 5, respectively) did not yield PCR products. The event ASR368 (lanes 1-3) produced the expected 896 bp band. The PCR products from these reactions were subjected to DNA sequencing. The combined overlapping DNA sequences establish the sequence of the 5' insert to plant junction (Figure V.11).

Figure V.12 shows the result of the PCR confirmation of the 3' insert to plant junction. As expected, the non-transformed control and the no template control (lanes 4 and 5, respectively) did not yield PCR products. The event ASR368 (lanes 1-3) produced the expected 474 bp band. The PCR products from these reactions were subjected to DNA sequencing. The combined overlapping DNA sequences confirm and establish the sequence of the 3' insert to plant junction (Figure V.12).

A.2.j. PCR analysis and sequence confirmation of the genetic element organization and the *cp4 epsps* coding regions

The overlapping PCR products generated from the DNA insert in event ASR368 were subjected to DNA sequencing to further confirm the organization of the elements within the insert and the *ctp2-cp4 epsps* coding regions. The consensus sequence representing the insert in event ASR368, generated by compiling numerous sequencing reactions performed on the two PCR products that spanned the length of the insert, is shown in Figure V.13. The DNA sequence of the insert contains 6639 bases with base one equal to base 212 of PV-ASGT08, and base 6639 equal to 6850 in plasmid PV-ASGT08 (Figure IV.1). Both of the PCR products also contained creeping bentgrass genomic DNA flanking the insert, which is identical to that reported in Figures V.11 and V.12.

The deduced amino acid sequences of the CTP-CP4 EPSPS proteins encoded in event ASR368 were identical to the deduced amino sequences of the CTP-CP4 EPSPS proteins encoded in plasmid vector PV-ASGT08.

A.2.k. Conclusions

Roundup Ready creeping bentgrass event ASR368 was produced by particle acceleration technology using a linear DNA segment from plasmid PV-ASGT08 containing two *cp4 epsps* gene expression cassettes. Event ASR368 contains one insertion of the integrated DNA located on a 10 kb *Hin*d III restriction fragment. This insert contains one copy of the segment used in transformation. The individual genetic components in each of the two CP4 EPSPS gene expression cassettes in the integrated DNA are intact. The genome of event ASR368 does not contain any detectable plasmid backbone DNA including, *ori* or the *nptII* coding sequences. Sequences of the 5' and 3' ends of the insert were determined by genome walking and confirmed by PCR amplification and nucleotide sequencing. In addition, the *ctp2-cp4 epsps* coding regions were confirmed to be identical to those in plasmid PV-ASGT08. These data establish that only the expected full-length CTP2-CP4 EPSPS protein should be encoded by the insert in event ASR368. In addition, the genetic stability of the inserted DNA was demonstrated by Southern blot analysis on genomic DNA of the R0, F1 and F2 generations of event ASR368.

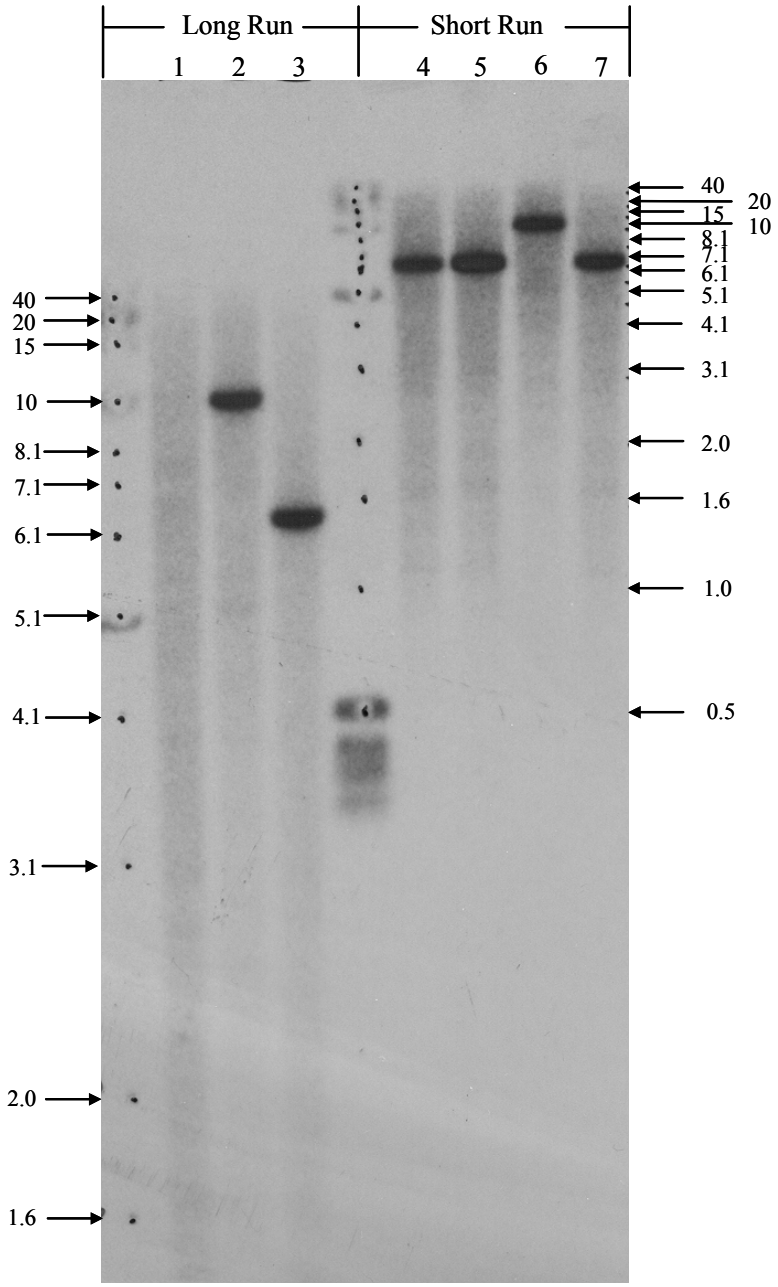

Figure V.1. Southern blot analysis of event ASR368: insert probe #1

Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with restriction enzymes. The blot was probed with ^{32}P-labeled insert probe #1. Lane designations are as follows: Lane 1 99061R/990028 (*Nde* I/*Sma* I); Lane 2 ASR368 (*Hin*d III); Lane 3 ASR368 (*Nde* I/*Sma* I); Lane 4 99061R/990028 + 10 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 5 99061R/990028 + 20 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 6 ASR368 (*Hin*d III); Lane 7 ASR368 (*Nde* I/*Sma* I).

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

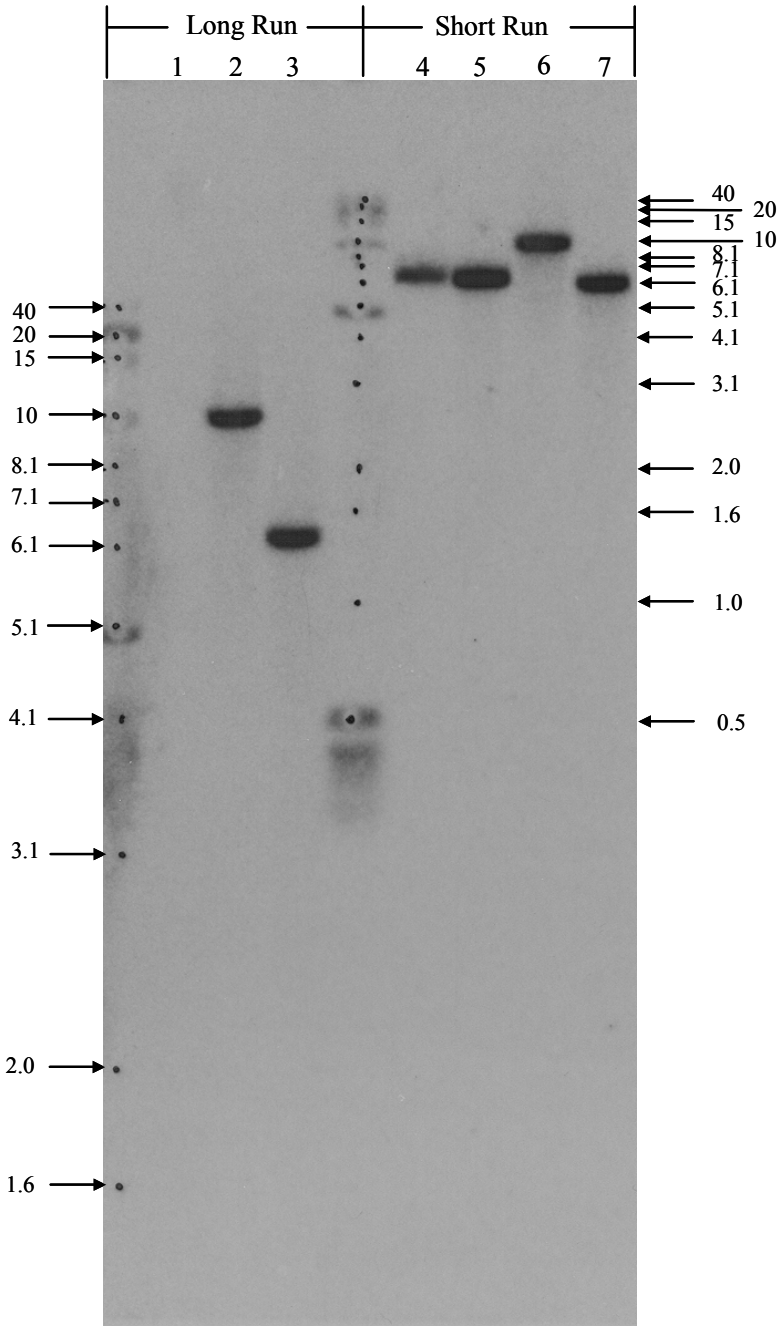

Figure V.2. Southern blot analysis of event ASR368: insert probe #2
Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with restriction enzymes. The blot was probed with ^{32}P-labeled insert probe #2. Lane designations are as follows: Lane 1 99061R/990028 (*Nde* I/*Sma* I); Lane 2 ASR368 (*Hin*d III); Lane 3 ASR368 (*Nde* I/*Sma* I); Lane 4 99061R/990028 + 10 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 5 99061R/990028 + 20 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 6 ASR368 (*Hin*d III); Lane 7 ASR368 (*Nde* I/*Sma* I).
→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

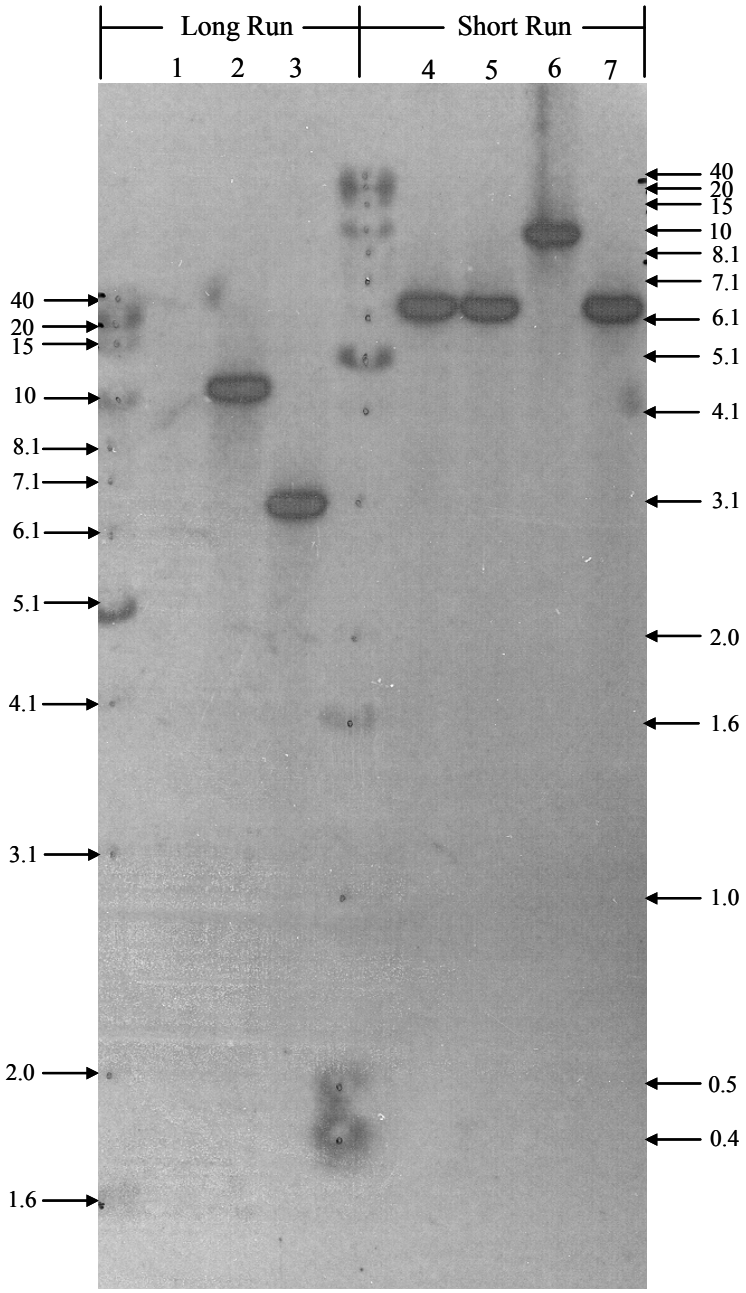

Figure V.3. Southern blot analysis of event ASR368: *ctp2-cp4 epsps* probe
Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with restriction enzymes. The blot was probed with ^{32}P-labeled *ctp2-cp4 epsps*. Lane designations are as follows: Lane 1 99061R/990028 (*Nde* I/*Sma* I); Lane 2 ASR368 (*Hin*d III); Lane 3 ASR368 (*Nde* I/*Sma* I); Lane 4 99061R/990028 + 10 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 5 99061R/990028 + 20 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 6 ASR368 (*Hin*d III); Lane 7 ASR368 (*Nde* I/*Sma* I).
→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

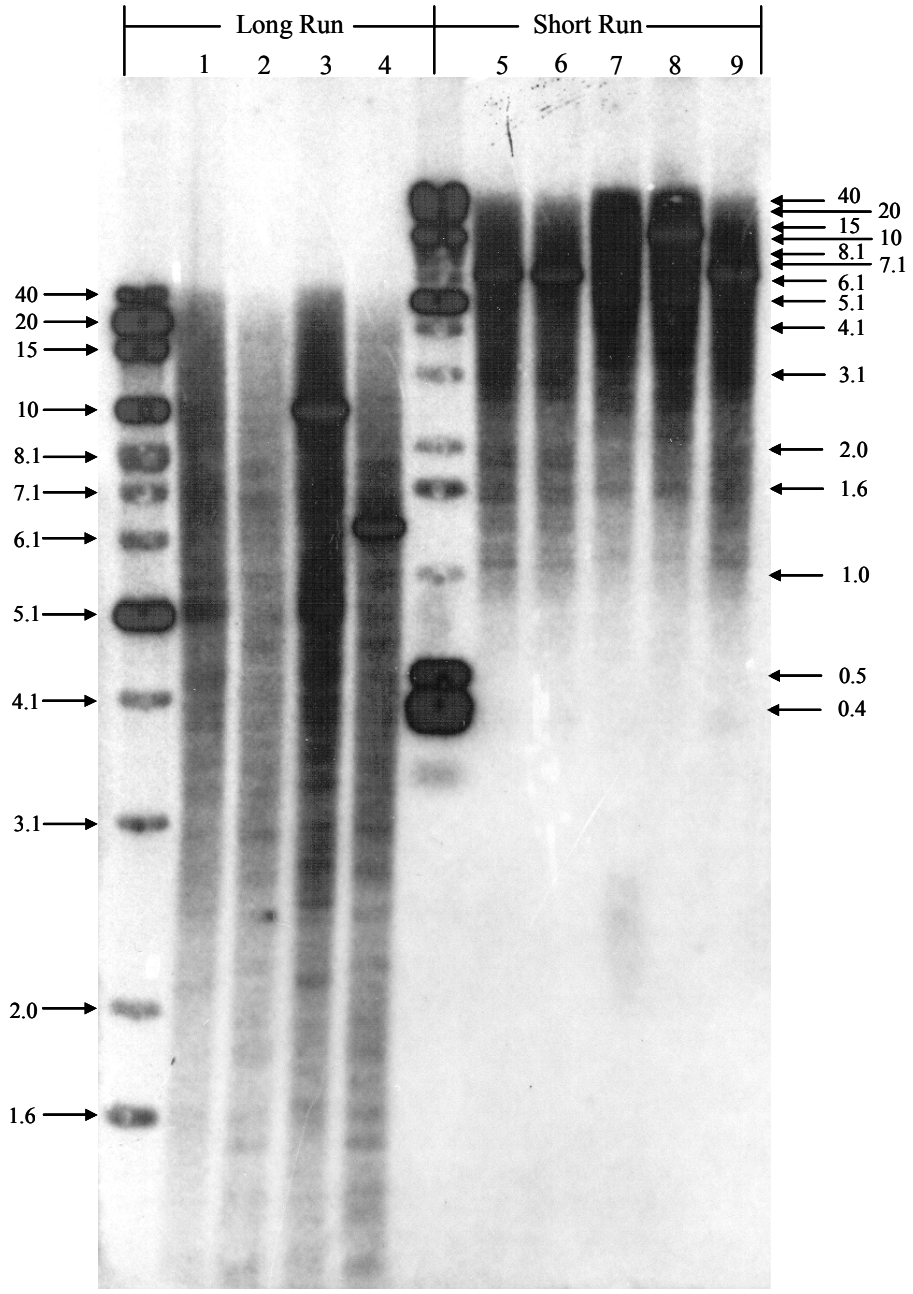

Figure V.4. Southern blot analysis of event ASR368: P-ract/intron probe
Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with restriction enzymes. The blot was probed with ^{32}P-labeled rice actin promoter and intron. Lane designations are as follows: Lane 1 99061R/990028 (*Hin*d III); Lane 2 99061R/990028 (*Nde* I/*Sma* I); Lane 3 ASR368 (*Hin*d III); Lane 4 ASR368 (*Nde* I/*Sma* I); Lane 5 99061R/990028 + 10 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 6 99061R/990028 + 20 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 7 99061R/990028 (*Hin*d III); Lane 8 ASR368 (*Hin*d III); Lane 9 ASR368 (*Nde* I/*Sma* I).
→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

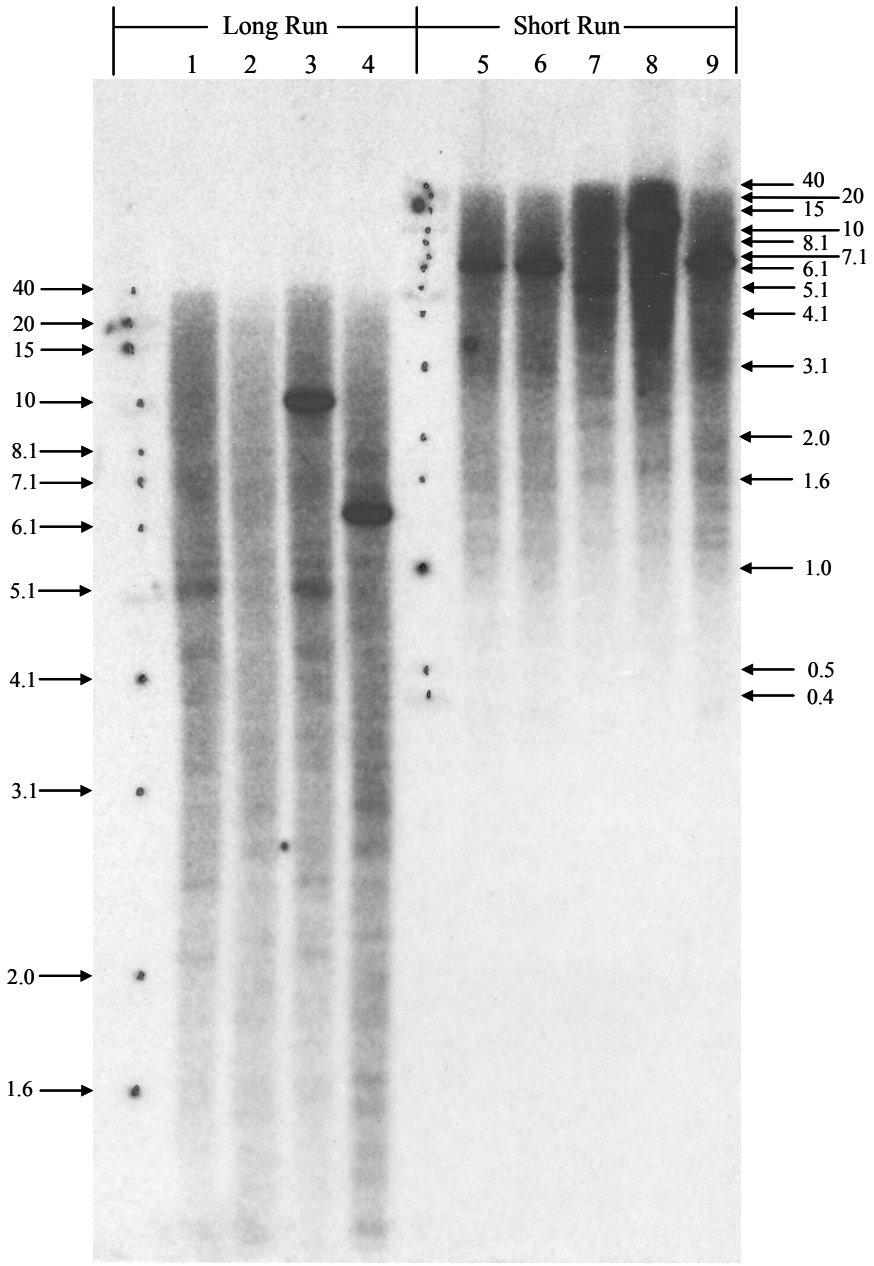

Figure V.5. Southern blot analysis of event ASR368: P-ract probe

Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with restriction enzymes. The blot was probed with ^{32}P-labeled rice actin promoter and intron. Lane designations are as follows: Lane 1 99061R/990028 (*Hin*d III); Lane 2 99061R/990028 (*Nde* I/*Sma* I); Lane 3 ASR368 (*Hin*d III); Lane 4 ASR368 (*Nde* I/*Sma* I); Lane 5 99061R/990028 + 10 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 6 99061R/990028 + 20 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 7 99061R/990028 (*Hin*d III); Lane 8 ASR368 (*Hin*d III); Lane 9 ASR368 (*Nde* I/*Sma* I).

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

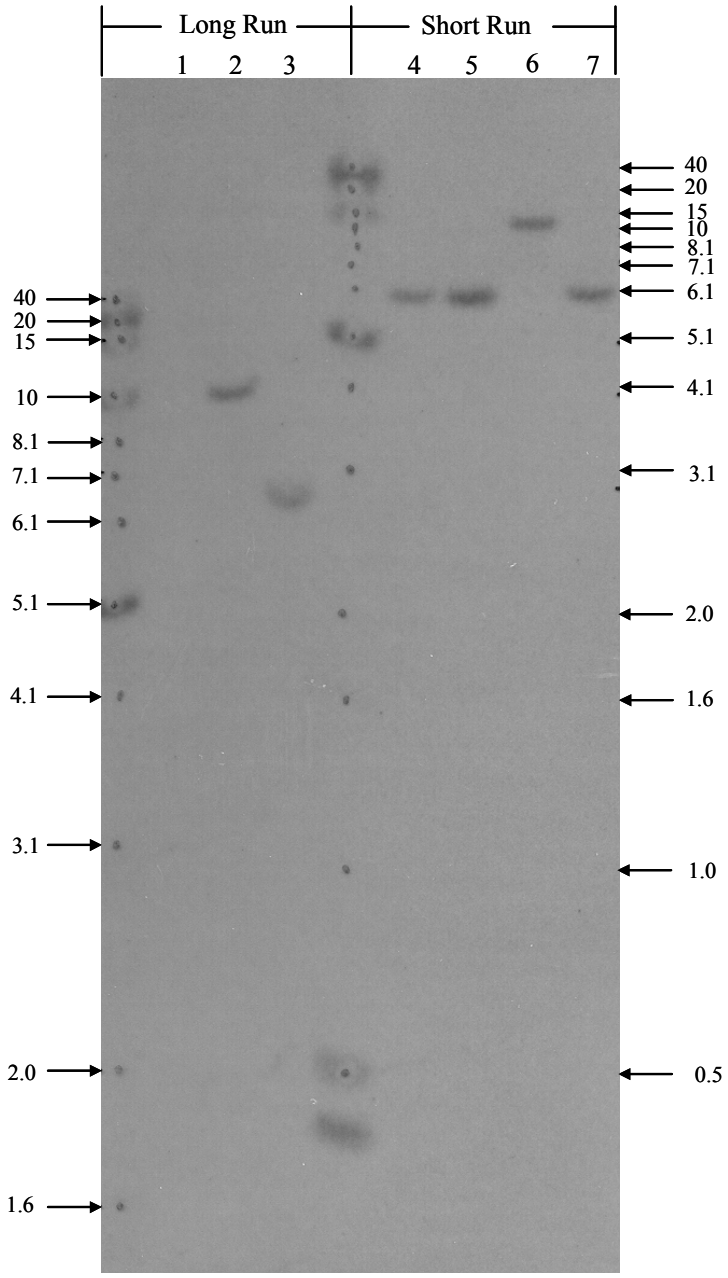

Figure V.6. Southern blot analysis of event ASR368: NOS 3' polyadenylation sequence probe

Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with restriction enzymes. The blot was probed with ^{32}P-labeled NOS 3' polyadenylation sequence. Lane designations are as follows: Lane 1 99061R/990028 (*Nde* I/*Sma* I); Lane 2 ASR368(*Hin*d III); Lane 3 ASR368 (*Nde* I/*Sma* I); Lane 4 99061R/990028 + 10 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 5 99061R/990028 + 20 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 6 ASR368 (*Hin*d III); Lane 7 ASR368 (*Nde* I/*Sma* I).

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

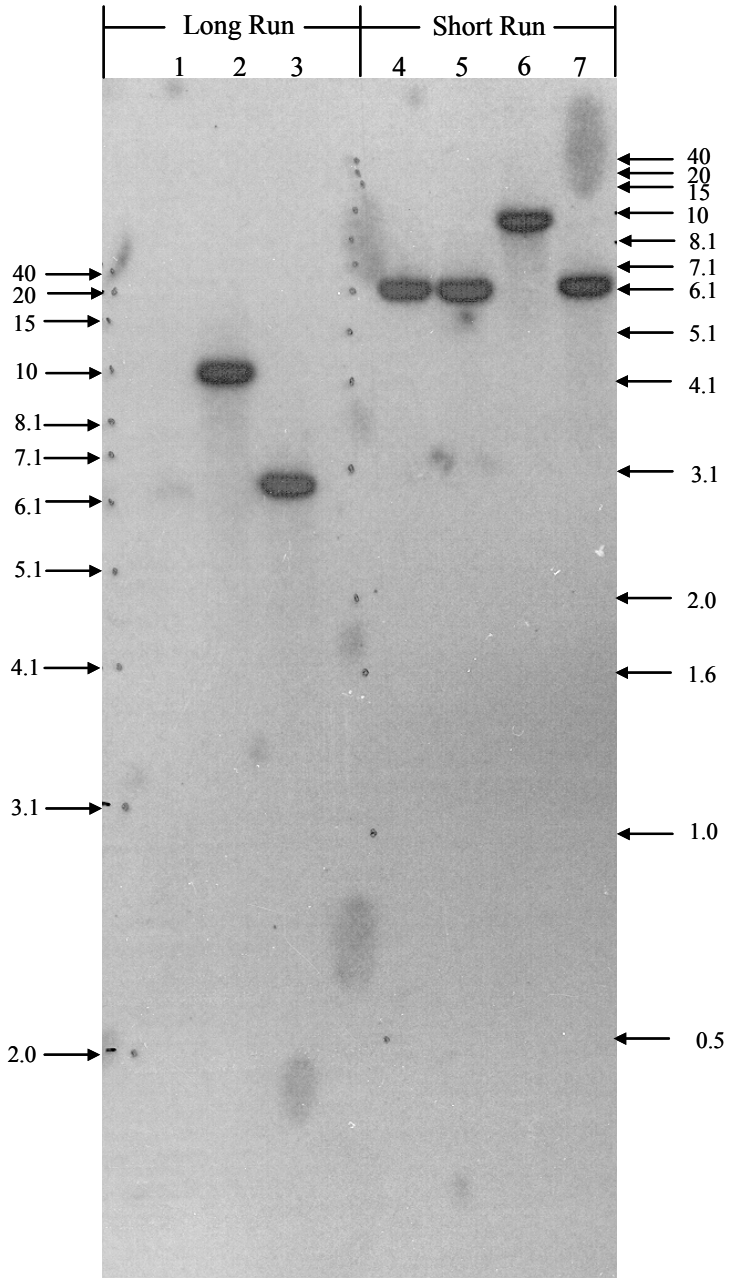

Figure V.7. Southern blot analysis of event ASR368: e35S/*Zm*HSP70 probe
Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with restriction enzymes. The blot was probed with ^{32}P-labeled enhanced CaMV promoter. Lane designations are as follows: Lane 1 99061R/990028 (*Nde* I/*Sma* I); Lane 2 ASR368 (*Hin*d III); Lane 3 ASR368 (*Nde* I/*Sma* I); Lane 4 99061R/990028 + 10 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 5 99061R/990028 + 20 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 6 ASR368 (*Hin*d III); Lane 7 ASR368 (*Nde* I/*Sma* I).
→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

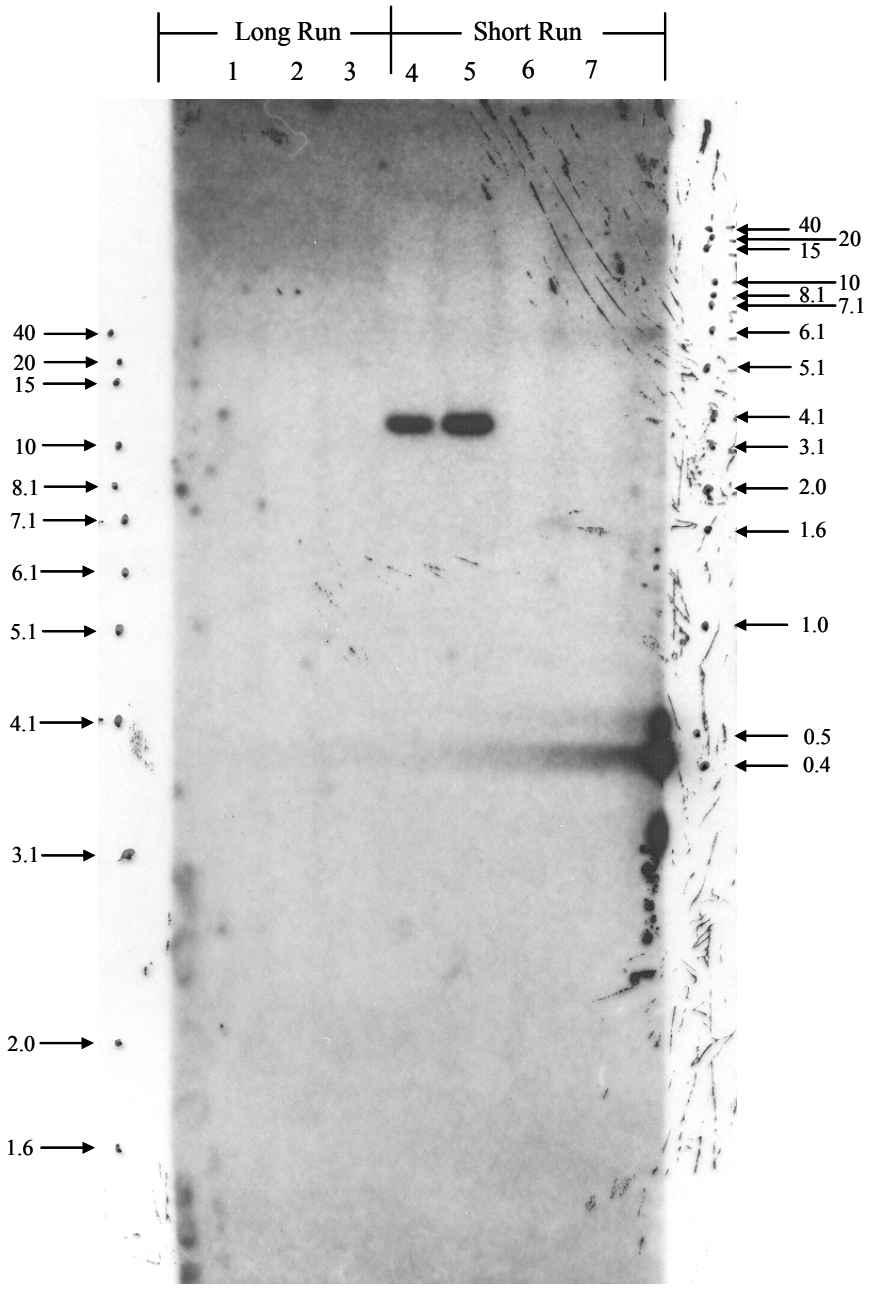

Figure V.8. Southern blot analysis of event ASR368: backbone probe
Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with restriction enzymes. The blot was probed with ^{32}P-labeled backbone. Lane designations are as follows: Lane 1 99061R/990028 (*Nde* I/*Sma* I); Lane 2 ASR368 (*Hin*d III); Lane 3 ASR368 (*Nde* I/*Sma* I); Lane 4 99061R/990028 + 10 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 5 99061R/990028 + 20 pg of PV-ASGT08 (*Nde* I/*Sma* I); Lane 6 ASR368 (*Hin*d III); Lane 7 ASR368 (*Nde* I/*Sma* I).
→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

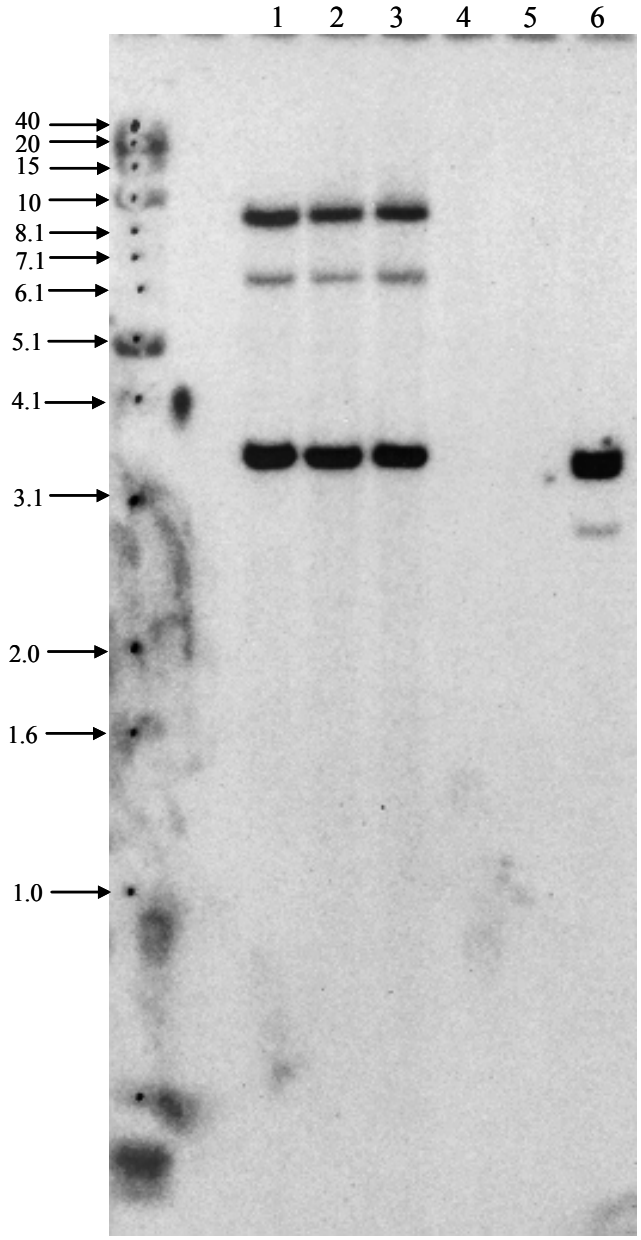

Figure V.9. Southern blot analysis of the genetic stability of event ASR368
Ten micrograms each of 99061R/990028 and ASR368 genomic DNA isolated from leaf tissue were digested with *Sph* I. The blot was probed with ^{32}P-labeled *ctp2-cp4 epsps*. Lane designations are as follows: Lane 1 ASR368 R0; Lane 2 ASR368 F1; Lane 3 ASR368 F2; Lane 4 V13-2-2; Lane 5 99061R/990028; Lane 6 99061R/990028 + 20 pg of PV-ASGT08.
→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

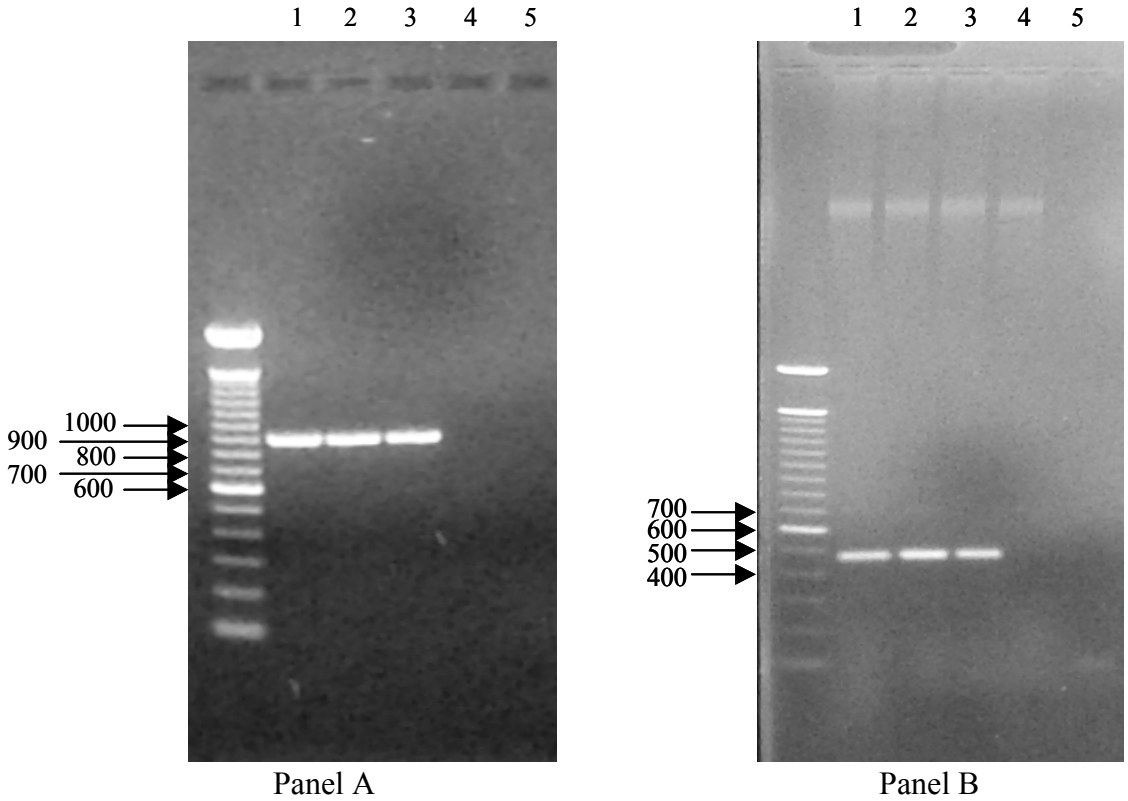

Figure V.10. PCR confirmation of the 5' and 3' border sequences of the event ASR368 insert

PCR was performed using primers specific to the 5' and 3' border sequences for event ASR368 on genomic DNA isolated from leaf tissue from 99061R/990028 (non-transformed control) and event ASR368. Panel A shows the results from the 5' border sequence confirmation. Panel B shows the results from the 3' border sequence confirmation. Lane designations are as follows:

Panel A – 5' border sequence verification
Lane 1 event ASR368
Lane 2 event ASR368
Lane 3 event ASR368
Lane 4 99061R/990028 non-transformed control
Lane 5 No template control

Panel B – 3' border sequence verification
Lane 1 event ASR368
Lane 2 event ASR368
Lane 3 event ASR368
Lane 4 99061R/990028 non-transformed control
Lane 5 No template control

→ Symbol denotes size of DNA, in base pairs, obtained with MW markers.

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Figure V.11. 5' Flanking sequence of the insert in event ASR368.
The underlined base pairs 1-637 represent the genomic DNA flanking the 5' end of the insert in event ASR368. Base pairs 638-896 are a portion of the rice actin promoter sequence. The PCR primers used to generate the PCR product are double underlined. The italicized, lower case sequence represents the oligonucleotide sequence that was not confirmed as part of this study because amplification and sequencing were performed with the same primer.

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Figure V.12. 3' Flanking sequence of the insert in event ASR368.
The base pairs 1-248 represent a portion of the NOS 3' polyadenylation sequence and the polylinker sequence. The underlined base pairs 249-474 represent the genomic DNA flanking sequence at the 3' end of the insert in event ASR368. The PCR primers used to generate the PCR product are double underlined. The italicized, lower case sequence represents the oligonucleotide sequence that was not confirmed as part of this study because amplification and sequencing were performed with the same primer.

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Figure V.13. DNA sequence of the insert in event ASR368.
Bases 1-6639 represent the DNA sequence of the transgene insert in creeping bentgrass event ASR368. Base one of the insert is equal to base 212 of plasmid PV-ASGT08, while base 6639 equals base 6850 in plasmid PV-ASGT08 (Figure IV.1).

B. Segregation data

B.1. Methods

A flowchart depicting the development and breeding history of event ASR368 and the progeny used to investigate genetic inheritance are presented in Figure V.14. Segregation data for nineteen populations derived from the reciprocal crosses made between F1 plants hemizygous for the *cp4 epsps* gene and derived from event ASR368 plants and elite parental plants are presented in Table V.1 (USDA # 00-22401n). Paired reciprocal crosses were conducted using pollination shoot bags (Lawson #411) to isolate 3-5 heads each of an F1 RR plant (hemizygous for the *cp4 epsps* gene) with an equal number of heads from a single elite parent plant (non-transgenic).

The inheritance of the introduced DNA in the progenies from the reciprocal F1 crosses was monitored phenotypically at the whole plant level by application of glyphosate at the two to three leaf stage in a greenhouse. Data from these analyses provide evidence of the number of loci, as well as the stability of the introduced DNA.

B.2. Results and discussion of segregation study

Statistical significance for the segregation data was determined using Chi square analysis. For these analyses a Chi square value (χ^2) was determined as follows: $\chi^2 = \sum [(|o-e|-0.5)^2/e]$ where o = observed frequencies for each class, e = expected frequencies for each class and 0.5 = Yates correction factor for Chi square analysis with one degree of freedom (df) (Little and Hills, 1978). The calculated Chi square value was compared to a table of Chi square values to determine whether the observed frequencies fit the expectation for a single insert at $p = 0.05$ and/or $p = 0.01$.

The Chi square analysis indicates that a single T-DNA insert in event ASR368 is integrated in the plant genome and is inherited as a single locus following a Mendelian one-locus model in a stable manner through several plant populations. These results are also consistent with the genetic analysis described in section V.A.2.h. which demonstrated the genetic stability of the transgene by Southern blot analysis of the R_0, F_1 and F_2 generations of event ASR368.

Creeping bentgrass is essentially an obligate outcrossing species as it is more likely to cross-pollinate than self-pollinate when exposed to other compatible pollen sources. The 1:1 Roundup resistant (RR) to Roundup sensitive (RS) progeny ratios identified among reciprocal cross progeny on page 110 indicate that cross-pollination between both non-transgenic plants and plants containing the *cp4 epsps* gene is more likely than self-pollination within these creeping bentgrass plants. The fact that reciprocal crosses provided similar 1:1 ratios indicates that both non-transgenic and transgenic plants are selfing and crossing in similar fashion. These results also support the conclusion that F1 RR progeny are hemizygous for the *cp4 epsps* gene.

Further discussion of the open-pollinating nature of creeping bentgrass can be found in section VI.E.1 of this petition. In greenhouse evaluations, viable seed set among all self-pollinated bentgrass genotypes was negligible in comparison to the number of viable seeds set upon open-pollination. These results reflect the self-incompatibility systems known to exist in the *Agrostis* genus.

Figure V.14. Source of genetic materials and crosses used to develop and investigate the genetic inheritance of ASR368.

Creeping bentgrass cv. Backspin

Conventional creeping bentgrass cultivar developed through the crossing of Penncross and several other collections of creeping bentgrass from U.S. and French golf courses.

1998 **B99061R/99028**

Non-transgenic, single seed selection from nontransgenic creeping bentgrass cv. Backspin.

- Transformation with PV-ASGT08L (via. particle bombardment)
- Selection and regeneration in tissue culture (glyphosate amended media)
- Explants transferred to greenhouse

1999 **Event ASR368 R0**

- Greenhouse assay for glyphosate resistance, plant selection with glyphosate
- Ro designation of RRCB event ASR368
- Transplants established in fall
- Transplants moved into isolated crossing blocks

2000 **ASR368 x Elite Parental Plants* (EPPs)**

EPPs are individual plants from conventional creeping bentgrass cultivars: Penneagle, Pennlinks, Providence, Southshore and Putter. These cultivars have been commercially available from 8 - 25 years.

- EPPs crossed with ASR368 (ASR368 used as pollen donor for *cp4 epsps* gene)
- F1 = Designation for seed harvested only from Elite Parent Plants
- R1 = Designation for seed harvested from ASR368 plants (maternal carried trait)
- Transplants established, whole plant selection with glyphosate
- F1 genotype Rr plants used in reciprocal crosses in the greenhouse

2001 **RRCBF1 x Elite Parental Plants (double recessive for trait)**

- Reciprocal crossing
- Seed harvested from both EPP and RRCB F1 plants
- Segregation analysis on inheritance of the phenotype
- F2 = Designation for seed harvested only from Elite Parent Plants

* Footnote regarding the genetic similarity of these varieties with Penncross, Crenshaw, Backspin and Penn A-4.

Table V.1. Segregation data and Chi square analysis of Roundup resistant (RR) and Roundup susceptible (RS) phenotypes recovered from progeny of reciprocal crosses involving F1 RR progeny derived from event ASR368 and elite parent plants

Population Designation		# of	RR[1]		deviation	-Yates	d^2	d^2	RS[2]		deviation	-Yates	d^2	d^2	Chi-	Signif.[3]
Female	male	Plants	obs.	expected	/(d)/	Corr. Fact.		expected	obs.	expected	/(d)/	Corr. Fact.		expected	Square	
10-1-8 x ASR368A	15-2-4	41	19	20.5	1.5	1	1	0.049	22	20.5	1.5	1	1	0.049	0.098	NS
15-2-4	10-1-8 x ASR368A	10	6	5	1	0.5	0.25	0.050	4	5	1	0.5	0.25	0.050	0.100	NS
10-1-8 x ASR368B	15-2-4	14	8	7	1	0.5	0.25	0.036	6	7	1	0.5	0.25	0.036	0.071	NS
15-2-4	10-1-8 x ASR368B	98	57	49	8	7.5	56.25	1.148	41	49	8	7.5	56.25	1.148	2.296	NS
14-2-6	13-2-2 x ASR368	282	131	141	10	9.5	90.25	0.640	151	141	10	9.5	90.25	0.640	1.280	NS
13-2-2 x ASR368	14-2-6	352	163	176	13	12.5	156.25	0.888	189	176	13	12.5	156.25	0.888	1.776	NS
14-2-6	13-2-2 x ASR368	64	28	32	4	3.5	12.25	0.383	36	32	4	3.5	12.25	0.383	0.766	NS
13-2-2 x ASR368	14-2-6	92	43	46	3	2.5	6.25	0.136	49	46	3	2.5	6.25	0.136	0.272	NS
13-2-2 x ASR368	15-2-9	37	20	18.5	1.5	1	1	0.054	17	18.5	1.5	1	1	0.054	0.108	NS
15-2-9	13-2-2 x ASR368	126	55	63	8	7.5	56.25	0.893	71	63	8	7.5	56.25	0.893	1.786	NS
15-2-9	13-2-2 x ASR368	51	23	25.5	2.5	2	4	0.157	28	25.5	2.5	2	4	0.157	0.314	NS
10-1-8 x ASR368C	10-1-7c	55	26	27.5	1.5	1	1	0.036	29	27.5	1.5	1	1	0.036	0.073	NS
10-1-7c	10-1-8 x ASR368C	66	35	33	2	1.5	2.25	0.068	31	33	2	1.5	2.25	0.068	0.136	NS
10-1-7c	13-2-2 x ASR368	25	11	12.5	1.5	1	1	0.080	14	12.5	1.5	1	1	0.080	0.160	NS
13-2-2 x ASR368	10-1-7c	91	47	45.5	1.5	1	1	0.022	44	45.5	1.5	1	1	0.022	0.044	NS
10-1-7v	15-2-5 x ASR368	22	10	11	1	0.5	0.25	0.023	12	11	1	0.5	0.25	0.023	0.045	NS
15-2-5 x ASR368	10-1-7v	94	41	47	6	5.5	30.25	0.644	53	47	6	5.5	30.25	0.644	1.287	NS
15-2-9	15-2-5 x ASR368	11	4	5.5	1.5	1	1	0.182	7	5.5	1.5	1	1	0.182	0.364	NS
15-2-5 x ASR368	15-2-9	39	27	19.5	7.5	7	49	2.513	12	19.5	1.5	1	0.5	0.026	2.538	NS

[1] RR= Roundup resistant, 2 RS= Roundup susceptible, 3 NS= not significant at p=0.05 (chi-square=3.84, 1 df).

C. Expression of the CP4 EPSPS protein

C.1. Introduction

A western blot analysis, using published methods (Harrison *et al*., 1996), was conducted to assess the equivalence of CP4 EPSPS protein produced in event ASR368 to the CP4 EPSPS protein produced in *E. coli* as well as to the protein produced in Roundup Ready soybeans.

This western blot (Figure V.15) shows that only one immuno-reactive protein of the expected apparent molecular weight is found in extracts of event ASR368. The light band at ~ 37 kD is a low abundance degradation product of full-length CP4 EPSPS protein. This conclusion is based upon two observations. First, regardless of the source of the CP4 EPSPS, the ~ 37 kD band is found only in those lanes that were loaded with purified CP4 EPSPS protein or extracts that include CP4 EPSPS protein. Lanes that contain control extract from non-transgenic bent grass (lane 3) and non-transgenic soy (lane 7) do not display a band of ~ 37 kD. Second, the intensity of the ~ 37 kD band is proportional to the loading of CP4 EPSPS. A visual comparison of lanes 1 and 2, or lanes 5 and 6 shows that lanes that were loaded with 2.5 ng of CP4 EPSPS (lanes 1 and 5) have a greater signal intensity at ~ 37 kD than the respective comparably loaded lanes (lanes 2 and 6) that contain 1.0 ng of CP4 EPSPS.

These results demonstrate that the CP4 EPSPS protein in event ASR368 is equivalent to that produced in *E. coli* and in commercial Roundup Ready soybeans. This justifies the use of the *E. coli*-produced CP4 EPSPS protein as a reference standard in the ELISA (Enzyme Linked Immuno-Sorbent Assay) assay used to estimate the levels of the CP4 EPSPS in event ASR368, as described in the following section.

C.2. Expression levels of CP4 EPSPS protein in Roundup Ready creeping bentgrass event ASR368

Levels of the CP4 EPSPS protein were estimated in event ASR368 forage samples collected from replicated field sites during the 2000 - 2001 growing season. The field release sites were: Marion County, OR; Union County, OH; Clinton County, IL; and Ottawa County, MI. Field production was conducted using agronomic practices typical of the commercial cultivation of creeping bentgrass and under environmental conditions representative of geographical regions where creeping bentgrass could be grown. Therefore, data generated on lines of event ASR368 are representative of the CP4 EPSPS protein levels that would be expected in commercially grown creeping bentgrass containing the Roundup Ready trait.

Grass forage samples collected from event ASR368 and the non-transformed parental control line (B99061R) consisted of all shoot tissue approximately one inch above the soil surface. The forage samples were analyzed using a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (Harlow and Lane, 1988) to estimate the level of CP4 EPSPS protein present in forage tissue. The ELISA consisted of a

monoclonal anti-CP4 EPSPS antibody as the capture antibody and a polyclonal anti-CP4 EPSPS conjugated to horseradish peroxidase as the detection antibody. A horseradish peroxidase substrate, TMB (3,3',5,5' tetramethylbenzidene), was added for color development. The CP4 EPSPS protein levels in forage extracts were quantified by comparison of the sample absorbance (OD) to the absorbance produced by a range of concentrations of the *E. coli*-produced CP4 EPSPS reference standard. The CP4 EPSPS protein standard was purified from an *E. coli* strain expressing the *Agrobacterium* sp. strain CP4 EPSPS gene. The protein standard has been previously characterized (Harrison *et al*., 1993).

The CP4 EPSPS protein levels (corrected for assay bias) estimated in creeping bentgrass forage samples for event ASR368 are summarized in Table V.2. The average CP4 EPSPS protein level in forage tissue, collected across the growing season from event ASR368 was 68.6 µg/g fwt, with a standard deviation of 16.9 µg/g fwt. Additionally, the CP4 EPSPS protein levels were comparable across the growing season, ranging from 77.1 µg/g fwt (standard deviation 12.7 µg/g fwt) as the average of the first sampling time point to 64.1 µg/g fwt (standard deviation 16.2 µg/g fwt) as the average of the final sampling time point. All of the control samples were below the LOD of 9.9 µg/g fwt. In summary, the grand average CP4 EPSPS protein level, across timepoints, was estimated to be 68.6 µg/g fwt, with a standard deviation of 16.9 µg/g fwt in event ASR368 forage.

In summary, the average CP4 EPSPS protein level, across timepoints, was estimated to be 68.6 µg/g fwt, with a standard deviation of 17.3 µg/g fwt in Roundup Ready bentgrass forage generated from an n=60; five timepoints, four field sites and three replications.

Table V.2. CP4 EPSPS protein levels in plant forage tissues collected from event ASR368 produced in U.S. field trials in the years 2000 and 2001.

Tissue Type	Forage[1] (Young Leaf) Timepoint 1	Forage[1] (OSL2) Timepoint 2	Forage[1] (OSL3) Timepoint 3	Forage[1] (OSL4) Timepoint 4	Forage[1] (OSL5) Timepoint 5
Average CP4 EPSPS Protein Level (µg/g fwt)[2, 3]	77.1	69.7	66.6	65.6	64.1
Standard Deviation[3]	12.7	20.4	13.6	21.4	16.2
Range[4]	63.6 – 105.2	33.6 – 104.5	39.8 – 86.5	25.9 – 97.1	42.9 – 92.9
B99061R	<LOD[5]	<LOD[5]	<LOD[5]	<LOD[5]	<LOD[5]

1 Forage samples consisted of the whole aerial portion of the plant, minus the roots, and harvested at the late vegetative growth (pseudo-erect) stage. Samples were collected at five sampling time-points labeled as: Young Leaf, OSL2 (~ 100 days after planting), OSL3 (~ 330 days after planting), OSL4 (~ 390 days after planting) and OSL5 (~480 days after planting).

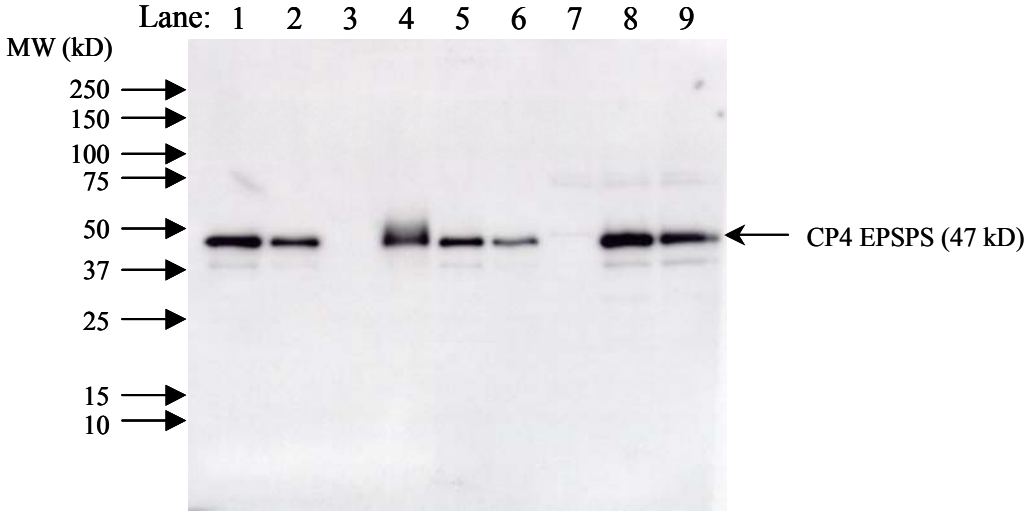
2 Protein levels are expressed as micrograms (µg) of protein per gram (g) fresh weight of tissue (fwt) and have been corrected for overall method bias.

3 Forage Analyses for ASR368: the average and standard deviation were calculated for each timepoint from the analyses of three replicate from four field sites (n=12).

4 Minimum and maximum values from the analyses of samples across all sites for each tissue type.

5 The level of the CP4 EPSPS protein in the control line (B99061R) was below the limit of detection (LOD) for all forage samples (9.9 µg/g fwt).

Figure V.15. Western blot showing the equivalence of CP4 EPSPS protein expressed by E. coli Roundup Ready soybean and Roundup Ready creeping bentgrass event ASR-368



Lane	Description	Amount of Total Protein
1	Control bentgrass extract spiked with 2.5 ng *E. coli* CP4 EPSPS standard	5.0 µg control extract and 2.5 ng standard
2	Control bentgrass extract spiked with 1 ng *E. coli* CP4 EPSPS standard	5.0 µg control extract and 1 ng standard
3	Control bentgrass extract (Backspin)	5.0 µg control extract
4	ASR-368 bentgrass extract	5.0 µg
5	*E. coli* CP4 EPSPS standard	2.5 ng
6	*E. coli* CP4 EPSPS standard	1 ng
7	Control soybean extract (A1900)	5.0 µg
8	Control soybean extract spiked with 2.5 ng *E. coli* CP4 EPSPS standard	5.0 µg control extract and 2.5 ng standard
9	A5602 RR soybean extract	5.0 µg

An aliquot of the 1X Laemmli samples for bentgrass and soybean were diluted 10-fold before analysis because of the high expression level of CP4 EPSPS.

D. Lack of toxicants in creeping bentgrass

Creeping bentgrass varieties have been grown on U.S. golf courses for more than 100 years and for the past 75 years have been grown almost exclusively for seed production in the Willamette Valley of Oregon (Schoth, 1930). Hence, the plant has a long history of safe use in both golf course and seed production environments. Humans do not consume creeping bentgrass. Nonetheless, a literature search was performed to identify toxicants in creeping bentgrass, but none were discovered. The CP4 EPSPS protein is also not considered a toxicant or allergen (OECD, 1999; Harrison *et al.*, 1996).

There are no documented clinical reports of allergic reactions specific to *Agrostis stolonifera*. In addition, this species is not considered to be one of the grasses of clinical significance based on known allergens that cause clinical reactions (Suphioglu, 2000). Creeping bentgrass may have the ability to elicit an allergic reaction from a susceptible individual due to cross-reactive allergens from different grasses (Suphioglu, 1993). However, creeping bentgrass has been grown almost exclusively in the Willamette Valley of Oregon for more than 75 years and there has been no specific record of an occurrence in the scientific literature. In addition, creeping bentgrass is maintained at a height of ½ inch or less on golf courses, which is below the height at which pollination occurs (Lush, 1988). Consequently, since (1) CP4 EPSPS is not homologous with known airborne allergens, (2) there is a long history of exposure to creeping bentgrass pollen by seed producers in the Willamette Valley of Oregon without specific reports of allergic reactions to the crop, (3) creeping bentgrass does not pollinate on golf courses and (4) other than tolerance to glyphosate, Roundup Ready and conventional creeping bentgrass are essentially the same, the likelihood that allergy to Roundup Ready creeping bentgrass will be a concern is remote.

E. Compositional and quality component analyses

Compositional analyses were conducted on leaf forage samples from ASR368, the non-transformed parent, B99061R and three conventional varieties produced in replicated plots established at sites in Marion County, OR, Union County, OH, Ottawa County, MI and Clinton County, IL. Single samples of four additional conventional varieties were also included to establish commercial ranges and tolerance intervals.

McCrimmon (1994), Youngberg and Vough (1977) and the Atlas of Nutritional Data on United States and Canadian Feeds (1972) were consulted to determine the appropriate compositional analytes and their range in creeping bentgrass straw. Comparative analyses of proximates (protein, fat, ash and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, minerals (calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc) and carbohydrates by calculation were performed. In all, 17 different components were analyzed to assess the composition of event ASR368.

There were few differences between the measured analytes of event ASR368, its non-transformed parent (B99061R) and the commercial varieties for the analytes evaluated at

all locations (Table V.3). The few differences between event ASR368 and B99061R are presented in Table V.4. All of these differences were only observed at one or two of the four sites. These components were not statistically significantly different at the other sites and fell well within the range observed for the commercial non-transgenic varieties evaluated. Therefore, it is concluded that event ASR368 is compositionally equivalent to and as safe and nutritious as the forage produced from other bentgrass varieties currently on the market.

Although humans do not consume creeping bentgrass, the straw and screenings that remain after the seed is cleaned are used minimally as animal feed. This data and information was provided to the FDA as per the FDA Food Policy, on September 13, 2002. This policy recommends that key compositional components of genetically modified plant varieties be performed prior to their commercial use as a food or animal feed.

Table V.3. Statistical summary of combined sites creeping bentgrass forage proximate, fiber and mineral content of forage for Roundup Ready creeping bentgrass event ASR368, non-transformed parental control line (B99061R) and several commercial cultivars.

Analytical Component	ASR368[1] Mean ± S.E. (Range)	B99061R Mean ± S.E. (Range)	Difference (Test Event ASR368 minus Control Line B99061R) Mean ± S.E. (Range)	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Ash (% DW)	10.17 ± 2.02 (6.19 - 16.81)	10.35 ± 2.02 (6.33 - 17.34)	-0.18 ± 1.06 (-3.24 - 3.17)	-2.48,2.13	0.869	(6.11 - 21.43) [0,19.83]
Carbohydrates (% DW)	68.51 ± 2.68 (62.39 - 75.00)	68.34 ± 2.68 (60.97 - 73.78)	0.17 ± 1.47 (-3.00 - 3.59)	-3.04,3.38	0.908	(59.16 - 78.12) [56.74,87.86]
Moisture (% FW)	71.05 ± 1.84 (65.80 - 76.30)	72.08 ± 1.84 (68.80 - 78.40)	-1.03 ± 0.80 (-4.80 - 1.20)	-2.77,0.72	0.225	(65.70 - 78.00) [64.30,79.89]
Protein (% DW)	17.43 ± 1.52 (14.31 - 21.54)	17.74 ± 1.52 (13.79 - 22.28)	-0.31 ± 0.93 (-2.67 - 1.91)	-2.34,1.71	0.741	(10.70 - 22.81) [9.57,21.25]
Total Fat (% DW)	3.89 ± 0.28 (2.44 - 5.65)	3.57 ± 0.28 (2.24 - 4.93)	0.32 ± 0.34 (-2.49 - 2.47)	-0.35,0.99	0.346	(2.36 - 6.37) [0.52,6.69]
Fiber						
Acid Detergent Fiber (% DW)	24.34 ± 1.27 (19.47 - 29.53)	24.07 ± 1.27 (21.48 - 26.06)	0.27 ± 0.84 (-2.89 - 4.30)	-1.57,2.11	0.756	(21.73 - 32.84) [14.45,37.01]
Crude Fiber (% DW)	18.07 ± 0.83 (16.70 - 20.49)	17.43 ± 0.83 (14.65 - 19.78)	0.64 ± 0.72 (-0.80 - 2.69)	-0.92,2.20	0.389	(15.45 - 23.48) [11.79,27.27]
Neutral Detergent Fiber (% DW)	48.07 ± 1.35 (42.34 - 54.27)	45.43 ± 1.35 (41.64 - 50.90)	2.64 ± 1.50 (-4.21 - 8.95)	-0.62,5.90	0.102	(41.60 - 53.52) [40.90,55.81]
Mineral						
Calcium (100g/kg DW)	0.052 ± 0.0075 (0.036 - 0.076)	0.055 ± 0.0075 (0.037 - 0.10)	-0.0026 ± 0.0044 (-0.026 - 0.0033)	-0.011,0.0062	0.559	(0.029 - 0.096) [0.030,0.081]

[1] Number represents average across the four sites and the range of values observed.
[2] With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table V.4. Summary of statistically significant results for the comparison of component levels for event ASR368 vs. non-transformed parental control line (B99061R) and several commercial cultivars.

Analytical Component	Units	Mean ASR368	Mean B99061R	Mean Diff.(ASR368 minus B99061R) % of B99061R	Mean Diff.(ASR368 minus B99061R) Signif. (p-Value)	ASR368 (Range)	Commercial (Range)	Commercial (Tolerance Int.)[1]
					Site OR			
Fiber								
Neutral Detergent Fiber	% DW	48.33	42.77	13.00	0.020	(45.48 - 50.00)	(41.60 - 53.52)	[40.90,55.81]
					Site IL			
Fiber								
Neutral Detergent Fiber	% DW	52.76	45.68	15.50	<0.001	(51.38 - 54.27)	(41.60 - 53.52)	[40.90,55.81]
Proximate								
Moisture	% FW	67.93	71.83	-5.43	0.026	(65.80 - 70.70)	(65.70 - 78.00)	[64.30,79.89]
Mineral								
Phosphorus	100g/kg DW	0.035	0.040	-13.46	0.046	(0.032 - 0.038)	(0.020 - 0.040)	[0,0.055]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

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VI. Agronomic Evaluation

As discussed in Section II of this petition, *A. stolonifera* has been studied for decades and much is known of its biology, life history and pest potential. This body of knowledge establishes familiarity with the species and serves as a baseline for the variability common to creeping bentgrasses for a particular plant characteristic. This baseline can also be used to help compare the plant pest potential of Roundup Ready creeping bentgrass event ASR368 with other commercial creeping bentgrass cultivars (Hokanson *et al.,* 1999).

To help establish greater familiarity with event ASR368 and to better understand its plant pest or weed potential, more than 90 experiments were performed between 1999 and 2003 at 65 locations representing the northern or cool, southern or warm and transition climate zones of turfgrass adaptation. These experiments describe the biology, morphology and life history of plants derived from this event. The results of these experiments are presented in eight chapters within Section VI, organized to represent a different aspect of the creeping bentgrass biology and life history. The stages of the life cycle and the plant characteristics evaluated at each stage that could contribute to ASR368 posing a plant pest risk are provided in Figure VI.1. These include:

1) Establishment via seed.

2) Establishment via vegetative stolons.

3) Relative growth in several environments under competitive and non-competitive environments representing cool season, warm season and transition zone turfgrass growing areas.

4) Flowering period: initiation of flowering, beginning of anthesis and anthesis duration.

5) Pollen biology: size and viability/longevity.

6) Fecundity: duration of seed set and components of seed yield.

7) Seed longevity, dormancy, germination energy and seedling vigor.

8) Quantitative and/ or qualitative assessments of a number of botanical structures or characteristics such as: flag leaf, panicle, floret, ligule, stolons, nodes, bud leaf vernation and leaf venation.

Experimental Comparators

ASR368 was chosen from among more than four hundred transformation events because of its commercially acceptable agronomic and phenotypic characteristics and tolerance to Roundup IT&O herbicides. Using a forward breeding strategy, as discussed in Section

II.A.2.b, clones of the ASR368 R0 generation were crossed with a number of Elite Parent Plants to develop the R1 and F1 progeny populations (Figure V.14). As a result of this unique breeding strategy, each individual plant of an ASR368 seedling population is genotypically and phenotypically distinct yet representative of *A. stolonifera*.

A number of different comparators were employed for ASR368 in the experiments presented in this section. Due to the forward breeding process and the potential for somaclonal variation among plants regenerated from tissue culture (Evans *et al.,* 1984; Fluminhan *et al.,* 1996; Muller *et al.,* 1990), near isogenic or tissue culture lines were considered of limited value as comparators for ASR368. Therefore, the following were also employed as comparators: (1) commercial cultivars that represent the range of *A. stolonifera* agronomic and phenotypic characteristics, (2) the Elite Parent Plants (EPPs), which were selected from commercially available *A. stolonifera* cultivars developed before 1994 and were crossed with ASR368 R0 generation plants to produce the R1, F1 and F2 progeny populations and/or, (3) null segregant or "Roundup Susceptible" plants from which non-transgenic populations were developed. The commercial cultivars, EPPs and null segregants were considered more appropriate non-transgenic organism comparators for assessing the plant pest risk of ASR368 than either near-isogenic or tissue culture lines.

The ASR368 treatments and comparators employed in each experiment presented in this section are depicted in Table VI.1 and include:

R0: Initial generation event ASR368 plants derived directly from transformation, subsequently regenerated in tissue culture and further maintained by vegetative propagation. Identified as ASR368 R0 in the experiments presented in this section.

R1: Second progeny population of ASR368 plants resulting from the hybridization of an ASR368 R0 mother plant and pollen from a population of Elite Parent Plants. Identified as ASR368 R1 in the experiments presented in this section.

F1: Second progeny population of ASR368 plants resulting from the hybridization of an Elite Parent Plant mother plant and pollen from R0 ASR368. Identified as ASR368 F1 in the experiments presented in this section.

F2: Third progeny population of ASR368 plants resulting from the hybridization of an Elite Parent Plant mother plant and pollen from F1 ASR368. Identified as ASR368 F2 in the experiments presented in this section.

RR: A Roundup Ready or glyphosate tolerant plant that has inherited the *cp4 epsps* gene.

RS: A glyphosate susceptible plant identified among the segregating ASR368 F1 or R1 progeny population. These "RS" plants or "null segregants" did not inherit the *cp4 epsps* gene. RS has also been used to refer to other non-transgenic conventional plants used as comparators in experiments.

B99061R/990028 (non-transgenic tissue culture line): Developed from randomly selected non-transgenic embryogenic callus regenerated in tissue culture and further maintained by vegetative propagation. This tissue culture line was included as an additional comparator in many of the experiments in which ASR368 R0 was evaluated. B99061R/990028 is neither an isogenic nor near-isogenic line to ASR368. Identified as B99061R in the experiments presented in this section.

Elite Parent Plants (EPPs): The EPPs are individual plants selected based on progeny turf trials from the traditionally bred and commercially available creeping bentgrass cultivars Penneagle, Pennlinks, Providence, Putter and Southshore. These varieties, which were developed prior to 1994, were chosen for intercrossing with ASR368 because they possess numerous characteristics desired by the golf course industry.

Backspin: Backspin was developed by Texas A&M University through the intercrossing of selections from Penncross and three other non-transgenic collections of creeping bentgrass obtained from golf courses in the United States and France. The variety was commercially introduced in 1999. B99061R and ASR368 were developed from callus tissue derived from separate seeds selected from Backspin.

Penncross: Penncross was released nearly 50 years ago and is the most widely planted creeping bentgrass cultivar in the United States. The cultivar was developed by breeders at Pennsylvania State University from existing creeping bentgrass turf strains. It is a parent of many currently commercial cultivars and is used as a standard cultivar for variety comparison in the National Turf Evaluation Program (http://www.ntep.org/).

Penn A-4: Penn A-4 was developed by Pennsylvania State University turfgrass breeders and released in 1995. The parents for this cultivar were selected from existing greens likely planted to the creeping bentgrass cultivars Penncross, Penneagle and Pennlinks. Penn A-4 exhibits the highest shoot density of existing cultivars and has been in the National Turf Evaluation Program for more than five years (http://www.ntep.org/).

Crenshaw: Crenshaw was developed by Texas A&M University in the 1990s and was commercially released in 1993. The variety was selected for heat tolerance, is highly susceptible to dollarspot disease and is widely planted in the Southeast United States. Crenshaw has been in the National Turf Evaluation Program trials for more than 10 years (http://www.ntep.org/).

Other bentgrass cultivars: In a number of experiments several other commercial bentgrass cultivars served as comparators for ASR368, e.g. SR1020, Seaside, and Penneagle (*A. stolonifera*), Highland and Trust dryland bentgrass (*A. castellana*), Bardot and SR7100 colonial bentgrass (*A. capillaris*) and Streaker and Reton redtop bentgrass (*A. gigantea*).

This collection of non-transgenic genotypes is generally representative of the genotypic variability of *A. stolonifera* and are appropriate comparators to assess whether Roundup Ready creeping bentgrass event ASR368 has been altered in a biologically meaningful

manner. With these comparators the weed or plant pest potential of event ASR368 can be assessed through agronomic and phenotypic evaluations.

The experiments presented in Section VI were conducted under field conditions and/or in a greenhouse or poly-house. The controlled conditions possible in a greenhouse enable varieties to be developed and evaluated throughout the year in environments representative of that naturally inhabited by *A. stolonifera* or in which it is agriculturally produced. Greenhouses also enabled the flowering characteristics of event ASR368 to be studied without the isolation required under field conditions. In addition, if one or more characteristics of plant growth or development were changed as a result of the plant transformation process, the change(s) would likely be expressed in a consistent manner across multiple environments, including those maintained in a greenhouse or poly-house.

Finally, in order to simplify the text of Section VI, unless otherwise noted, "genotype" is used to represent a population of individual genotypes that comprise a commercial creeping bentgrass cultivar or a population of ASR368 R1, F1 or F2 progeny as well as single genotypes such as the ASR368 R0 or B99061R.

Figure VI.1. Summary of creeping bentgrass life cycle, organization of Section VI and studies performed to assess the agronomic characteristics of event ASR368.

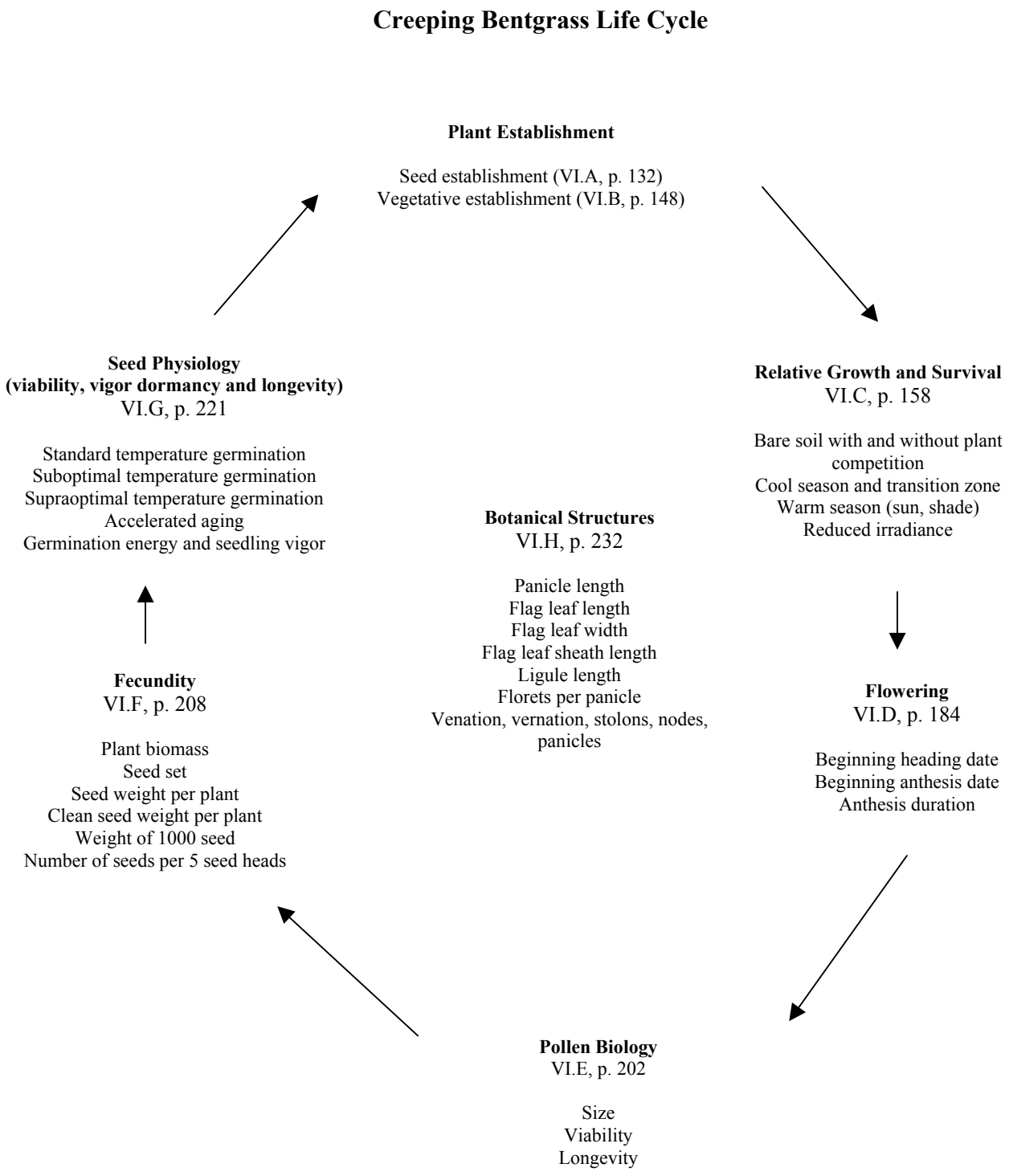


Table VI.1. Studies performed from 2000 – 2003 to assess the agronomic and phenotypic characteristics of ASR368.

Study	Gen.[1]	Controls[2]	Location[3]	Pg.
Plant Establishment				
Bare soil - seed	R1	A-4, BS, CR, PC	MA, OR	135
Competition - seed	R1	A-4, BS, CR, PC	MA, OR	138
Vegetative – stolons	R1	A-4, CR, PC, SR1020, NS	KY (GH)	148
Vegetative – stolons	R1	BS, PE	OR (PH)	149
Vegetative – stolons (field/GH)	F1, F2	PC, A-4, CR, EPP	KY, OH, OR, AL	151
Relative Growth				
Bare soil	R0	A-4, B99, CR, PC	IL, MI, OH, OR	160
Competition, cool season (R0)	R0	A-4, B99, CR, PC	NJ, OH, OR	168
Competition, cool season (F1)	F1	A-4, B99, BS, CR, PC, HL, SR7, ST	OH	171
Competition, warm season (shade)	F1	A-4, B99, BA, BS, CR, PC, HL, SR7, ST	AL	176
Competition, warm season (sun)	F1	A-4, B99, BA, BS, CR, PC, HL, SR7, ST	AL	178
Bare soil, reduced irradiance	F1	A-4, B99, BS, CR, PC, HL, SR7, ST	MI	180
Flowering[4]				
Flowering - greenhouse	R0, F1	A-4, B99, CR, PC	IA (GH)	184
Flowering - greenhouse	R0, F1, F2	A-4, BS, PC	IA (GH)	184
Flowering - field	R1	NS	WA (2)	195
Flowering – field	F1, F2	A-4, BS, CR	OR	195
Pollen Biology				
Pollen size, viability and long.	R0, F1	A-4, B99, CR, PC	IA (GH)	202
Pollen size, viability and long.	F2	A-4, BS, CR	IA (GH)	202
Fecundity				
Seed set, yield and veg. prod.	R0, F1	A-4, B99, CR, PC	IA (GH)	208
Seed set, yield and veg. prod.	R1	NS, EPP	WA (2)	212
Seed set, yield and veg. prod.	F1	A-4, BS, CR	OR	212
Seed set, yield and veg. prod.	F2	A-4, BS, CR	OR	212
Seed Physiology[5]				
SGT, SUB, SuOP, AAT, vigor	R1	NS, SR1020, HL	OR (GC, GH)	221
Comparative Botanical Characteristics[6, 7]				
Botanical characteristics	F1	EPP	OR (1), WA (1)	232
Botanical characteristics	F2	EPP	OR	232
Flower morphology	R0, F1,	A-4, B99, CR, PC	IA (GH)	238
Flower morphology	F2	A-4, BS, PC	IA (GH)	238

1 R0 = Initial generation ASR368 plants derived directly from transformation, R1 = seed harvested from ASR368 plants (maternal carried trait), F1 = seed harvested from EPP (ASR368 as pollen donor), F2 = seed harvested from EPP (ASR368 F1 or R1 as pollen donor)

2 Key to controls: A-4 = Penn A-4, BA = Bardot (colonial bentgrass), BS = Backspin, B99 = B99061R, CR = Crenshaw, NS = Null segregant, PC = Penncross, PE = Penneagle, HL = Highland bentgrass, SR7 = SR7100 (colonial bentgrass) ST = Streaker (redtop bentgrass), EPP = Elite parent plants (either individual plants of Penneagle, Pennlinks, Providence, Putter, Southshore or as a synthetic population of these creeping bentgrass cultivars)

3 Locations: AL = Alabama, IL = Illinois, , IA = Iowa, KY = Kentucky, MA = Massachusetts, MI = Michigan, NJ = New Jersey, OH = Ohio, OR = Oregon, WA = Washington, GH = greenhouse, GC = growth chamber, PH = poly-house

4 Flowering characteristics include: beginning head date, beginning anthesis date and anthesis duration

5 SGT = standard germination test, SUB = suboptimal germination test, SuOP = supraoptimal germination test and AAT = accelerated aging germination test, vigor = high and low germination and seedling vigor

6 Botanical characteristics include: panicle length, flag leaf length, flag leaf width, flag leaf sheath length, ligule length, florets per panicle and florets per spikelet, leaf venation, leaf vernation, anthers, stolons with nodes

7 Flower morphology: panicle length and florets/spikelet

A. Seed Establishment

The rate, breadth of environmental conditions and diversity of mechanisms by which a plant species is able to establish influences that species' potential to persist in the environment. As described in Section II.E.2, creeping bentgrass colonizes and prospers in environments with the potential for direct and firm contact of a seed or node with soil and little environmental stress, i.e. good mineral nutrition, water availability and abundant light (Hunt *et al.,* 1987). Seed establishment of ASR368 was asessed in experiments depicted in this section. The establishment of vegetative (detached stolon) and whole plants will be reported in sections VI.B and VI.C, respectively.

Studies were conducted to determine the relative ability of seed from ASR368 R1 and four commercial creeping bentgrass cultivars (Backspin, Crenshaw, Penn A-4 and Penncross) to establish and persist in bare soil (non-competitive) and mature turf (competitive) environments. These studies were conducted in Marion County, Oregon during 2000, 2001 and 2002, and Franklin County, Massachusetts during 2000 - 2001 under USDA notification numbers 00-224-01n and 01-228-02n.

A.1. Marion County, Oregon and Franklin County, Massachusetts (2000 – 2001)

A.1.a. Experimental methods

Plant Material

Five genotypes of creeping bentgrass seed material were used in this study. ASR368 R1 seed (expected to segregate 1:1 for Roundup resistant and Roundup sensitive phenotypes) and four commercial cultivars: Penncross, Penn A-4, Crenshaw, and Backspin. Prior to planting, the percent germination of each seed lot was determined using the standard AOSA seed germination test (AOSA, 1998). The germination percentages are presented in Table VI.A.1 and the number of potential seedlings per plot are presented in Table VI.A.2. The number of potential seedlings per plot of the 25 seed planted per genotype was calculated using the germination data.

Table VI.A.1. Creeping bentgrasses and germination percentages for the 2000 – 2001 seed establishment studies in Marion County, Oregon and Franklin County, Massachusetts.

Genotype	% Germination[1]
ASR368 RR	88
Penncross	94
Penn A-4	96
Crenshaw	93
Backspin	96

[1] Mean % germination determined by AOSA standard methods on 4 replications of 100 seed subsamples each from the same seed lots.

Table VI.A.2. Creeping bentgrasses and number of potential seedlings per plot in the 2000 – 2001 seed establishment studies in Marion County, Oregon and Franklin County, Massachusetts.

Genotype	Number of Potential Seedlings Per Plot[1]
ASR368 RR	22
Penncross	24
Penn A-4	24
Crenshaw	23
Backspin	24

[1] Number of potential seedlings was calculated using the formula: (% germination x 25 seed planted = number of potential seedlings per plot).

Field Plantings (2000 – 2001)

Two plantings were made at the Marion County, Oregon location. A fall planting was made on October 27, 2000 at the initiation of the rainy season and a spring planting was made on March 21, 2001 prior to the end of the rainy season. In Franklin County, Massachusetts, one fall planting was made on September 20, 2000.

Experimental design

Two levels of competition were used during establishment at both the Oregon and Massachusetts locations.

The Oregon study included: (1) a bare soil test plot void of vegetation and (2) a vegetative plot consisting of a mature plant canopy (4-6 inches) of 'Brigade' hard fescue. The bare soil plot was fumigated, tilled and irrigated prior to study initiation on October 27, 2000. The competitive plots were established on the same dates as the bare soil plots. No additional tillage or mowing occurred once the studies were initiated. Winter and spring rains in Oregon provided sufficient soil moisture for germination of both the fall and the spring planting dates.

The Massachusetts location also included two contrasting levels of competition for establishment: (1) a bare soil test plot site stripped of the existing vegetation with a sod cutter, and (2) a vegetative site covered with a mature 10-year old Kentucky bluegrass, fine fescue and perennial ryegrass turf stand with a plant canopy height of three to five inches under low maintenance. This experiment was established on September 20, 2000 as an irrigated and non-irrigated fall planting within each competition level. However, no irrigation events were needed after the initial irrigation as the fall season received ample amounts of natural precipitation. A frost occurred within 10 days of initiation at this site. Dormancy of the surrounding turfgrass was observed by mid-October and the test site was covered with snow and ice by the end of October, which subsequently killed all seedlings.

The plots at both study sites were arranged in a completely randomized design with three replications within each of the four combinations of competition level (bare soil or vegetated) and season (spring or fall in Oregon) or irrigation (+ or – in Massachusetts) regimes. Each plot was 1 m x 1 m square. A 30 cm x 30 cm square was centered within each plot as the test area. A total of 25 seeds were added to each plot on a uniform 6 cm spacing using a grid pattern as a guide in the 30 cm x 30 cm square. The seed was dropped onto the surface of the bare soil or the fescue-covered site without incorporation or pressing into the soil to simulate natural seed dissemination conditions.

Data Collection

Monthly seedling and plant counts were taken throughout the duration of the experiments in Oregon. The total number of potential seedlings per plot was calculated by multiplying the germination percentage determined for each seed line by the number of seed sown (Table VI.A.1). Survivability was then calculated as the number of seedlings established divided by the total number of potential seedlings (Table VI.A.1).

In Massachusetts, seedling and plant counts were made on October 5 and 12, 2000. Observations were not recorded again until the following spring as plant dormancy was observed by mid-October and persistent low temperatures coupled with snow and ice followed for the remainder of the winter. Data collection resumed in March 2001 and all plots were observed monthly through September.

Statistical Methods

Fisher's Exact Test ($\alpha = 0.05$) was used to compare the survivability of ASR368 versus each commercial cultivar in Oregon. A Least Significant Difference analysis ($\alpha = 0.05$) was used to examine the potential for significant differences between means in the Massachusetts data.

A.1.b. Results of 2000 – 2001 seed establishment studies

A.1.b.1. Bare soil seedling establishment - Oregon

Fall Planting

Seedling survival rates for seed of ASR368 R1 and the four commercial cultivars, Backspin, Crenshaw, Penn A-4 and Penncross from October 2000 through September 2001 are presented in Table VI.A.3. No seedlings were observed in any plot from October through February. Survival rates fluctuated over time. Fluctuation in seedling number suggests that: (1) individual seed germination occurred over time, (2) seedlings were difficult to locate at earlier stages of development and/or (3) seedlings may have germinated and subsequently perished. A maximum of 30% field survival (Crenshaw 9/7/01) was noted for any creeping bentgrass genotype planted in non-competitive bare soil plots during the fall. Throughout the duration of the study, ASR368 R1 seed or seedling establishment was significantly lower than Crenshaw on all but one date. In comparison to the other commercial cultivars ASR368 R1 establishment tended to be not significantly less or within the range of the commercial creeping bentgrass cultivars evaluated ($\alpha = 0.05$). These differences would not be expected to increase the plant pest potential of ASR368.

Spring Planting

Table VI.A.4 depicts the spring 2001 seedling survival rates in Oregon for ASR368 R1 and the four commercial cultivars: Backspin, Crenshaw, Penn A-4 and Penncross. Survival rates fluctuated over time similar to what was observed for the fall sowing dates. The fluctuation in plant numbers again suggests: 1) that individual seed germination occurred over time, (2) seedlings were difficult to locate at earlier stages of development and/or (3) seedlings may have germinated, established and subsequently perished. Throughout the duration of the study, ASR368 R1 seed or seedling establishment was not significantly different from the commercial creeping bentgrass cultivars evaluated ($\alpha = 0.05$). Less than 9% maximum field survival from spring sowing was observed among any creeping bentgrass genotype in non-competitive environments.

Table VI.A.3. Fisher's Exact Test comparison of survivability of ASR368 R1 seed and seedlings with several commercial creeping bentgrass cultivars planted on bare soil during fall 2000 in Marion County, Oregon.

Obs. Date	Genotype	Survivability	Difference[1]	95% Conf. Int.[2]		P - value
3/12/01	ASR368	0.015				
	Backspin	0.043	-0.028	-0.084	0.028	0.6645
	Crenshaw	0.153	-0.138	-0.226	-0.049	*0.0071
	Penn A-4	0.069	-0.054	-0.120	0.011	0.2513
	Penncross	0.057	-0.042	-0.104	0.020	0.4035
04/01/01	ASR368	0.091				
	Backspin	0.086	0.005	-0.090	0.101	1.0000
	Crenshaw	0.264	-0.173	-0.296	-0.050	*0.0141
	Penn A-4	0.222	-0.131	-0.250	-0.013	0.0586
	Penncross	0.157	-0.066	-0.176	0.044	0.3646
05/12/01	ASR368	0.076				
	Backspin	0.129	-0.053	-0.154	0.048	0.4669
	Crenshaw	0.278	-0.202	-0.324	-0.080	*0.0034
	Penn A-4	0.222	-0.146	-0.262	-0.031	*0.0286
	Penncross	0.229	-0.153	-0.270	-0.036	*0.0237
06/07/01	ASR368	0.167				
	Backspin	0.128	0.039	-0.092	0.170	0.7689
	Crenshaw	0.250	-0.083	-0.218	0.051	0.3217
	Penn A-4	0.236	-0.069	-0.203	0.064	0.4237
	Penncross	0.186	-0.019	-0.147	0.109	0.9485
07/04/01	ASR368	0.076				
	Backspin	0.157	-0.081	-0.188	0.025	0.2268
	Crenshaw	0.236	-0.160	-0.277	-0.043	*0.0173
	Penn A-4	0.208	-0.133	-0.246	-0.019	*0.0462
	Penncross	0.171	-0.096	-0.205	0.013	0.1516
08/07/01	ASR368	0.068				
	Backspin	0.157	-0.089	-0.202	0.024	0.2623
	Crenshaw	0.236	-0.168	-0.291	-0.045	*0.0319
	Penn A-4	0.229	-0.161	-0.301	-0.021	0.0594
	Penncross	0.213	-0.145	-0.283	-0.006	0.0913
09/07/01	ASR368	0.045				
	Backspin	0.143	-0.097	-0.200	0.005	0.1742
	Crenshaw	0.292	-0.246	-0.368	-0.125	*0.0014
	Penn A-4	0.229	-0.184	-0.318	-0.050	*0.0221
	Penncross	0.143	-0.097	-0.200	0.005	0.1742

[1] Difference between ASR368 survival and commercial cultivar survival
[2] 95% Confidence Interval of difference
* Significant difference detected between specified commercial cultivar and ASR368 R1 seed based on Fishers exact test (α =0.05)

Table VI.A.4. Fisher's Exact Test comparison for survivability of ASR368 R1 seed and seedlings with several commercial creeping bentgrass cultivars planted on bare soil during spring 2001 in Marion County, Oregon.

Obs. Date	Genotype	Survivability	Difference[1]	95% Conf. Int.[2]		P-value
04/01/01	ASR368	0.000				
	Backspin	0.000	0.000	0.000	0.000	1.0000
	Crenshaw	0.000	0.000	0.000	0.000	1.0000
	Penn A-4	0.014	-0.014	-0.041	0.013	1.0000
	Penncross	0.000	0.000	0.000	0.000	1.0000
05/12/01	ASR368	0.000				
	Backspin	0.000	0.000	0.000	0.000	1.0000
	Crenshaw	0.000	0.000	0.000	0.000	1.0000
	Penn A-4	0.042	-0.042	-0.088	0.004	0.2783
	Penncross	0.057	-0.057	-0.112	-0.003	0.1345
06/07/01	ASR368	0.000				
	Backspin	0.000	0.000	0.000	0.000	1.0000
	Crenshaw	0.042	-0.042	-0.098	0.015	0.3503
	Penn A-4	0.056	-0.056	-0.108	-0.003	0.1423
	Penncross	0.071	-0.071	-0.132	-0.011	0.0673
07/04/01	ASR368	0.045				
	Backspin	0.086	-0.040	-0.130	0.050	0.6768
	Crenshaw	0.028	0.018	-0.055	0.090	0.9797
	Penn A-4	0.056	-0.010	-0.091	0.071	1.0000
	Penncross	0.043	0.003	-0.075	0.080	1.0000
08/07/01	ASR368	0.023				
	Backspin	0.043	-0.020	-0.085	0.045	0.9967
	Crenshaw	0.042	-0.019	-0.091	0.053	1.0000
	Penn A-4	0.014	0.009	-0.043	0.061	1.0000
	Penncross	0.057	-0.034	-0.104	0.036	0.7145
09/07/01	ASR368	0.045				
	Backspin	0.043	0.003	-0.075	0.080	1.0000
	Crenshaw	0.000	0.045	-0.016	0.107	0.4520
	Penn A-4	0.014	0.032	-0.036	0.099	0.6420
	Penncross	0.014	0.031	-0.036	0.099	0.6609

[1] Difference between ASR368 survival and commercial cultivar survival
[2] 95% Confidence Interval of difference

A.1.b.2. Bare soil seedling establishment - Massachusetts

No significant difference among genotypes in the number of germinated seedlings was observed on either the October 5 or 12, 2000 observation dates (LSD, $\alpha = 0.05$; Table VI.A.5). Although the study was continued past October 12, the winter of 2000/2001 was considered one of the worst with regard to overall cold injury of bentgrass at virtually all of the golf courses in the New England region (McCabe, 2001). Ice formation occurred in late December and remained until early April 2001. Germination and establishment ratings were resumed at this time but due to the severity of the weather, none of the seedlings established in the fall had survived. Germination and establishment ratings continued through September 2001 but no latent germination was noted for any genotype treatment. These results demonstrate that ASR368 R1 seed are no more likely to germinate, establish or persist under severe climatic conditions than the commercial cultivars.

Table VI.A.5. The average number of germinated seedlings at the first and second leaf stage on two October 2000 dates following the planting of ASR368 R0 and commercial cultivars on bare soil during September 2000 in Franklin County, Massachusetts[1]

Seed Line	# of Germinated Plants		# in First Leaf		# in Second Leaf	
	10/5	10/12	10/5	10/12	10/5	10/12
ASR368	12.0	16.5	1.7	12.2	0.0	0.0
Penncross	11.3	13.3	1.2	10.0	0.0	0.7
Penn A-4	11.8	14.0	1.7	11.2	0.0	0.2
Crenshaw	10.5	12.3	0.8	9.2	0.0	0.0
Backspin	13.8	14.8	1.2	10.0	0.0	0.0
LSD ($\alpha = 0.05$)	6.51	6.79	1.88	6.15	0.17	0.89

[1] Number of seedlings of 25 seed planted

A.1.b.3. Seed establishment in a competitive environment

Despite adequate rainfall, no seed of ASR368 R1 or the commercial cultivars were observed to establish in any competitive plot throughout the 12-month duration of the studies in Marion County, Oregon or Franklin County, Massachusetts as illustrated in Tables VI.A.6 through VI.A.8, respectively. These results reflect the general inability of creeping bentgrass plants to fully establish from seed and persist in competitive environments regardless if adequate moisture is present, as discussed in Section II.E.2 of this petition.

Table VI.A.6. Number of surviving ASR368 R1 and commercial cultivar plants observed in 2001 when sown from seed into a mature unmanaged sward of hard fescue during fall 2000 in Marion County, Oregon.

	Number of surviving seedlings							
	Date							
Seed Line	**10/1/00 - 2/2/01**	**3/12/01**	**4/1/01**	**5/12/01**	**6/7/01**	**7/4/01**	**8/7/01**	**9/7/01**
ASR368	0	0	0	0	0	0	0	0
Backspin	0	0	0	0	0	0	0	0
Crenshaw	0	0	0	0	0	0	0	0
Penn A-4	0	0	0	0	0	0	0	0
Penncross	0	0	0	0	0	0	0	0

Table VI.A.7. Number of surviving ASR368 R1 and commercial cultivar plants observed in 2001 when sown from seed into a mature unmanaged sward of hard fescue during spring 2001 in Marion County, Oregon.

	Number of surviving seedlings					
	Date					
Seed Line	**4/1/01**	**5/12/01**	**6/7/01**	**7/4/01**	**8/7/01**	**9/7/01**
ASR368	0	0	0	0	0	0
Backspin	0	0	0	0	0	0
Crenshaw	0	0	0	0	0	0
Penn A-4	0	0	0	0	0	0
Penncross	0	0	0	0	0	0

Table VI.A.8. Number of surviving ASR368 R1 and commercial cultivar plants observed in 2001 when sown from seed into a mature sward of Kentucky bluegrass and fine fescue during fall 2000 in Franklin County, Massachusetts*.

	Number of surviving seedlings						
	Date						
Seed Line	**10/1/00 – 2/2/01**	**4/5/01**	**5/12/01**	**6/10/01**	**7/6/01**	**8/16/01**	**9/15/01**
ASR368	0	0	0	0	0	0	0
Backspin	0	0	0	0	0	0	0
Crenshaw	0	0	0	0	0	0	0
Penn A-4	0	0	0	0	0	0	0
Penncross	0	0	0	0	0	0	0

* Plots inaccessible for observation during November, 2000 through March 2001 due to snow and ice cover.

A.2. Marion County, Oregon (2001 – 2002)

A.2.a. Experimental methods

Plant Material

Seed of the same five creeping bentgrass genotypes i.e., ASR368 R1, Backspin, Crenshaw, Penncross and Penn A-4 were used in the establishment of studies conducted in Marion County, Oregon in 2001 - 2002. The percentage germination of each seed lot was determined using the standard AOSA seed germination test (AOSA 1998). The germination percentage for each seed lot is provided in Table VI.A.9.

Field Plantings

Two plantings were made in Marion County, Oregon. A fall planting was made on September 27, 2001 and a spring planting was made on April 12, 2002. These plantings were chosen to evaluate seed establishment under four distinct environments i.e., irrigated and non-irrigated conditions in both competitive and non-competitive environments. The irrigated plots received at least one inch of water, total, either through supplemental irrigation and/or rainfall each week for eight weeks following planting. The non-irrigated plots received only natural rainfall.

Experimental design

Four different environments were employed: (1) an irrigated, non-competitive bare soil test plot void of vegetation, (2) non-irrigated, non-competitive bare soil test plot void of vegetation, (3) an irrigated vegetated and competitive plot in an open, but mature plant canopy (4-6 inches tall) of 'Brigade' hard fescue, (4) a non-irrigated vegetated and competitive plot in an open, but mature plant canopy (4-6 inches tall) of 'Brigade' hard fescue. Plots were prepared prior to planting and managed post-planting as previously described for the 2000 – 2001 studies in Section VI.A.1.a.

The studies were arranged in a randomized complete block design with three replications. Each plot was 1 m x 1 m square. A 30 cm x 30 cm square was centered within each plot as the test area. This 30 cm x 30 cm square subsequently received the seed drop. A seed count of 100/plot was used for all genotypes. A total of 100 seed were added to each plot in a random pattern within the 30 cm x 30 cm square. The seed was dropped onto the surface of the plots without incorporation or pressing into the soil to simulate natural seed dissemination conditions.

Data collection

Seedling plant counts per plot were recorded monthly throughout the duration of the study. A rank of plant size was based on estimated tiller counts for the fall planting for the months of May, June, July, September and October. All seedlings and plants

surviving the summer season were tested with a non-destructive immuno-assay strip test (Strategic Diagnostics Inc, Newark, DE) to determine the ratio of RR:RS on September 26, 2002. Plant diameter data was collected from all plots on September 27, 2002.

The total number of potential seedlings per plot was calculated by multiplying the germination percentage determined for each seed line by the number of seed dropped (Table VI.A.9). Survivability was then calculated as the number of seedlings established divided by the total number of potential seedlings. Data on the number of plants that established and persisted were recorded.

Table VI.A.9. RR to RS ratio, percent germination and expected seedlings per plot of transgenic and commercial creeping bentgrass cultivars used in the establishment and persistence study in Marion County, Oregon.

Genotype	RR: RS	Percent Germination	Expected Seedlings Per Plot[1]	
			RR+RS	RR
ASR 368 R1	49:51	88	88	43
Backspin	0:100	96	96	0
Penncross	0:100	94	94	0
Penn A-4	0:100	96	96	0
Crenshaw	0:100	93	93	0

[1] Calculated by: percent germination x 100 seed added to plot.

A.2.b. Results of 2001 – 2002 seed establishment studies

A.2.b.1. Bare soil seedling establishment – Oregon

Fall Planting 2001

Seed establishment in the fall 2001 irrigated study ranged from ca. 1% to a maximum of 8% (Penn A-4) over the twelve months in which observations were made. The rate of ASR368 R1 seed establishment tended to be less than but not significantly different from the commercial cultivars other than that of Penn A-4, which consistently established at a significantly greater rate (Table VI.A.10). Maximum survivability of any genotype at the end of the study in October 2002 was < 7% under these optimized conditions.

Percent establishment in the non-irrigated study ranged from less than 0.1% to ca. 10% for the genotypes tested (Table VI.A.11). ASR368 R1 seed establishment was not significantly different from Backspin throughout the entire study and to the other commercial cultivars for the first five months in which observations were taken (November 2001 to March 2002). Seed establishment of ASR368 R1 was significantly greater than Crenshaw in April through October, Penn A-4 in June through September

and Penncross in July (Table VI.A.11). Despite these transient differences, seed establishment was variable and low for each of the genotypes evaluated.

Spring Planting 2002

Seed establishment for the Spring 2002 irrigated and non-irrigated plantings was essentially zero (< 0.005%) for each of the genotypes tested, which precluded the performance of a statistical analysis. These results help to demonstrate the difficulty creeping bentgrass seed has establishing in bare soil regardless of whether adequate moisture is present.

A.2.b.2. Competitive establishment - Oregon

No seed of any genotype established in the fall or spring, irrigated or non-irrigated competitive plantings. These results are consistent with those of the 2000/2001 studies and the published scientific literature. Therefore, it is concluded that the ability of ASR368 seed to establish in an existing turf sward is not different from commercial creeping bentgrass cultivars representative of *A. stolonifera*.

Table VI.A.10. Fisher's Exact Test comparison for survivability of ASR368 R1 seed and seedlings with several commercial creeping bentgrass cultivars planted on bare soil and irrigated during fall 2001 in Marion County, Oregon.

Obs. Date	Genotype	Survivability	Difference[1]	95% Conf. Int.[2]		P-value
11/07/01	ASR368	0.015				
	Backspin	0.031	-0.016	-0.041	0.009	0.267
	Crenshaw	0.065	-0.049	-0.082	-0.017	0.004*
	Penn A-4	0.142	-0.127	-0.170	-0.084	<0.0001*
	Penncross	0.050	-0.034	-0.064	-0.005	0.030*
11/20/01	ASR368	0.072				
	Backspin	0.104	-0.032	-0.079	0.015	0.231
	Crenshaw	0.082	-0.010	-0.055	0.034	0.748
	Penn A-4	0.240	-0.168	-0.226	-0.109	<0.0001*
	Penncross	0.057	0.015	-0.026	0.056	0.489
11/26/01	ASR368	0.114				
	Backspin	0.153	-0.039	-0.096	0.017	0.211
	Crenshaw	0.125	-0.012	-0.066	0.043	0.694
	Penn A-4	0.243	-0.129	-0.192	-0.067	<0.0001*
	Penncross	0.121	-0.007	-0.061	0.047	0.894
12/17/01	ASR368	0.152				
	Backspin	0.167	-0.015	-0.076	0.046	0.643
	Crenshaw	0.143	0.008	-0.052	0.068	0.810
	Penn A-4	0.274	-0.123	-0.190	-0.056	0.001*
	Penncross	0.142	0.010	-0.050	0.069	0.809
12/27/01	ASR368	0.140				
	Backspin	0.181	-0.040	-0.101	0.021	0.205
	Crenshaw	0.140	0.000	-0.058	0.059	1.0000
	Penn A-4	0.264	-0.124	-0.190	-0.058	0.0003*
	Penncross	0.145	-0.005	-0.064	0.053	0.903
01/09/02	ASR368	0.167				
	Backspin	0.201	-0.035	-0.099	0.030	0.324
	Crenshaw	0.154	0.013	-0.049	0.074	0.726
	Penn A-4	0.278	-0.111	-0.180	-0.043	0.002*
	Penncross	0.152	0.014	-0.047	0.076	0.726
02/27/02	ASR368	0.155				
	Backspin	0.080	0.075	0.022	0.129	0.007*
	Crenshaw	0.108	0.048	-0.009	0.105	0.126
	Penn A-4	0.253	-0.098	-0.165	-0.032	0.004*
	Penncross	0.138	0.017	-0.042	0.076	0.629

[1] Difference between ASR368 survival and commercial cultivar survival
[2] 95% Confidence Interval of difference
* Significant difference detected between survivability of commercial cultivar and ASR368 R1 seed based on Fishers exact test (α =0.05)

Table VI.A.10. (contd.) Fisher's Exact Test comparison for survivability of ASR368 R1 seed and seedlings with several commercial creeping bentgrass cultivars planted on bare soil and irrigated during fall 2001 in Marion County, Oregon.

Obs. Date	Genotype	Survivability	Difference[1]	95% Conf. Int.[2]		P-value
03/28/02	ASR368	0.155				
	Backspin	0.076	0.079	0.026	0.132	0.005*
	Crenshaw	0.104	0.051	-0.005	0.108	0.095
	Penn A-4	0.253	-0.098	-0.165	-0.032	0.004*
	Penncross	0.135	0.021	-0.039	0.080	0.543
04/24/02	ASR368	0.155				
	Backspin	0.083	0.072	0.018	0.126	0.012
	Crenshaw	0.108	0.048	-0.009	0.105	0.126
	Penn A-4	0.250	-0.095	-0.161	-0.028	0.998
	Penncross	0.131	0.024	-0.035	0.083	0.464
05/24/02	ASR368	0.144				
	Backspin	0.080	0.064	0.011	0.117	0.020*
	Crenshaw	0.108	0.036	-0.019	0.092	0.2430
	Penn A-4	0.247	-0.103	-0.168	-0.037	0.003*
	Penncross	0.124	0.020	-0.037	0.077	0.531
06/24/02	ASR368	0.140				
	Backspin	0.094	0.046	-0.007	0.100	0.110
	Crenshaw	0.104	0.036	-0.019	0.091	0.237
	Penn A-4	0.247	-0.106	-0.171	-0.041	0.002*
	Penncross	0.121	0.020	-0.037	0.076	0.526
07/25/02	ASR368	0.140				
	Backspin	0.087	0.053	0.000	0.106	0.058
	Crenshaw	0.097	0.043	-0.011	0.098	0.143
	Penn A-4	0.243	-0.103	-0.168	-0.038	0.002*
	Penncross	0.128	0.012	-0.045	0.070	0.707
09/04/02	ASR368	0.061				
	Backspin	0.073	-0.012	-0.054	0.029	0.612
	Crenshaw	0.057	0.003	-0.036	0.043	1.0000
	Penn A-4	0.108	-0.047	-0.093	-0.001	0.066*
	Penncross	0.078	-0.017	-0.060	0.025	0.502
10/11/02	ASR368	0.068				
	Backspin	0.069	-0.001	-0.044	0.041	1.0000
	Crenshaw	0.039	0.029	-0.009	0.067	0.051
	Penn A-4	0.069	-0.001	-0.044	0.041	0.133
	Penncross	0.060	0.008	-0.033	0.049	0.730

[1] Difference between ASR368 survival and commercial cultivar survival
[2] 95% Confidence Interval of difference
* Significant difference detected between survivability of commercial cultivar and ASR368 R1 seed based on Fishers exact test (α =0.05)

Table VI.A.11. Fisher's Exact Test comparison for survivability of ASR368 R1 seed and seedlings with several commercial creeping bentgrass cultivars planted on bare soil and non-irrigated during fall 2001 in Marion County, Oregon.

Obs. Date	Genotype	Survivability	Difference[1]	95% Conf. Int.[2]		P-value
11/07/01	ASR368	0.008				
	Backspin	0.003	0.004	-0.008	0.017	0.609
	Crenshaw	0.004	0.004	-0.009	0.017	0.614
	Penn A-4	0.010	-0.003	-0.019	0.013	1.000
	Penncross	0.014	-0.007	-0.024	0.011	0.687
11/20/01	ASR368	0.019				
	Backspin	0.014	0.005	-0.016	0.026	0.743
	Crenshaw	0.025	-0.006	-0.031	0.018	0.773
	Penn A-4	0.042	-0.023	-0.051	0.006	0.144
	Penncross	0.028	-0.009	-0.035	0.016	0.579
11/26/01	ASR368	0.080				
	Backspin	0.052	0.027	-0.014	0.069	0.228
	Crenshaw	0.068	0.011	-0.033	0.055	0.626
	Penn A-4	0.083	-0.004	-0.049	0.042	0.878
	Penncross	0.067	0.012	-0.032	0.056	0.625
12/17/01	ASR368	0.080				
	Backspin	0.056	0.024	-0.018	0.066	0.308
	Crenshaw	0.065	0.015	-0.029	0.059	0.511
	Penn A-4	0.063	0.017	-0.026	0.060	0.507
	Penncross	0.074	0.005	-0.040	0.050	0.873
12/27/01	ASR368	0.095				
	Backspin	0.052	0.043	-0.001	0.086	0.070
	Crenshaw	0.061	0.034	-0.011	0.079	0.949
	Penn A-4	0.052	0.043	-0.001	0.086	0.982
	Penncross	0.060	0.034	-0.011	0.079	0.953
01/09/02	ASR368	0.087				
	Backspin	0.063	0.025	-0.019	0.069	0.330
	Crenshaw	0.072	0.015	-0.030	0.061	0.529
	Penn A-4	0.059	0.028	-0.015	0.072	0.250
	Penncross	0.064	0.023	-0.021	0.068	0.332
02/27/02	ASR368	0.061				
	Backspin	0.049	0.012	-0.026	0.050	0.577
	Crenshaw	0.029	0.032	-0.003	0.067	0.094
	Penn A-4	0.056	0.005	-0.034	0.044	0.856
	Penncross	0.053	0.007	-0.032	0.046	0.716

[1] Difference between ASR368 survival and commercial cultivar survival
[2] 95% Confidence Interval of difference

Table VI.A.11. (contd.) Fisher's Exact Test comparison for survivability of ASR368 R1 seed and seedlings with several commercial creeping bentgrass cultivars planted on bare soil and non-irrigated during fall 2001 in Marion County, Oregon.

Obs. Date	Genotype	Survivability	Difference[1]	95% Conf. Int.[2]		P-value
03/28/02	ASR368	0.061				
	Backspin	0.049	0.012	-0.026	0.050	0.577
	Crenshaw	0.025	0.036	0.001	0.070	0.054
	Penn A-4	0.052	0.009	-0.030	0.047	0.714
	Penncross	0.050	0.011	-0.027	0.049	0.580
04/24/02	ASR368	0.072				
	Backspin	0.066	0.006	-0.036	0.048	0.867
	Crenshaw	0.025	0.047	0.011	0.083	0.015*
	Penn A-4	0.052	0.020	-0.020	0.060	0.378
	Penncross	0.039	0.033	-0.006	0.071	0.132
05/24/02	ASR368	0.083				
	Backspin	0.056	0.028	-0.015	0.070	0.239
	Crenshaw	0.025	0.058	0.020	0.096	0.004*
	Penn A-4	0.045	0.038	-0.003	0.079	0.080
	Penncross	0.046	0.037	-0.004	0.079	0.083
06/24/02	ASR368	0.091				
	Backspin	0.069	0.021	-0.024	0.067	0.432
	Crenshaw	0.018	0.073	0.035	0.111	0.0002*
	Penn A-4	0.042	0.049	0.008	0.091	0.024*
	Penncross	0.043	0.048	0.006	0.090	0.025
07/25/02	ASR368	0.098				
	Backspin	0.059	0.039	-0.006	0.085	0.111
	Crenshaw	0.025	0.073	0.033	0.114	0.0005*
	Penn A-4	0.024	0.074	0.034	0.114	0.0002*
	Penncross	0.043	0.056	0.013	0.099	0.012*
09/04/02	ASR368	0.057				
	Backspin	0.035	0.022	-0.013	0.057	0.226
	Crenshaw	0.004	0.053	0.024	0.082	0.0002*
	Penn A-4	0.021	0.036	0.004	0.068	0.043*
	Penncross	0.028	0.028	-0.006	0.062	0.135
10/11/02	ASR368	0.049				
	Backspin	0.042	0.008	-0.027	0.042	0.688
	Crenshaw	0.004	0.046	0.019	0.073	0.001*
	Penn A-4	0.028	0.021	-0.011	0.054	0.265
	Penncross	0.032	0.017	-0.016	0.051	0.385

[1] Difference between ASR368 survival and commercial cultivar survival
[2] 95% Confidence Interval of difference
* Significant difference detected between survivability of commercial cultivar and ASR368 R1 seed based on Fishers exact test (α =0.05)

A.3. Overall conclusion for seed establishment studies

The percent germination for each of the seed lots used in these experiments ranged from 88% for the ASR368 R1 to 96% for the Penn A-4 and Backspin commercial varieties. These germination rates exceed the minimal germination threshold of 85% for Certified quality bentgrass seed production (Oregon State University, 2001). Despite this expected germination rate, establishment and field survival of all the creeping bentgrass cultivars seeded in either bare soil or existing vegetative stands was generally low, never exceeding 30%. ASR368 R1 seed establishment was significantly less than Penn A-4 in both the Marion County, Oregon bare soil fall 2000 study and fall 2001 irrigated study. In comparison to the other commercial cultivars, ASR368 R1 seed establishment tended to fall within the range of the other commercial cultivars in both the Oregon and Massachusetts bare soil studies. None of the genotypes were able to establish when seeded into existing turf.

As discussed in Section II.E.2, successful seedling establishment can be limited in existing turf swards due to either insufficient disturbance or excessive competition from the existing turfgrass population. Howe and Snaydon (1986); Jonsdottir (1991) and Bullock *et al.* (1994) noted that seedling recruitment into natural stands and pastures, respectively, was unsuccessful in augmenting *A. stolonifera* populations. Sweeney and Danneberger (1998) and Kendrick and Danneberger (2002) were also unable to demonstrate with molecular markers the success from direct inter-seeding *A. stolonifera* into an existing *A. stolonifera* stand utilized for golf putting green turf. The difficulty in establishment of *Agrostis* seedlings may be a consequence of the extremely small seed (0.07 mg seed^{-1}) size since small seed require a long duration of disturbance (reduced competition) and low stress for establishment and survival after germination (Cattani and Struik, 2001). Small seed may also preclude direct contact with soil in an existing vegetative stand.

Consequently, given the results from these experiments, which further confirm reports in the scientific literature, seed of ASR368 would not be expected to germinate, establish or persist in unmanaged competitive and non-competitive ecosystems differently from non-transgenic creeping bentgrasses. This supports a conclusion of no contribution to increased weed potential of ASR368 compared to commercial creeping bentgrass cultivars that are representative of *A. stolonifera* based on these seed establishment characteristics.

B. Vegetative Establishment

Creeping bentgrass can reproduce vegetatively through the production of stolons. Stolons are true lateral stems that contain nodes and internodes. The meristematic tissue present at the nodes can initiate new independent plants when they are disseminated into favorable conditions, which include intimate contact with soils, available moisture and low competition for resources. An enhanced ability to vegetatively establish could increase the persistence and plant pest potential of ASR368. This section compares the establishment ability of detached stolon sections containing nodes from plants of ASR368 R1, F1 and/or F2 progeny, ASR368 Roundup Susceptible segregants (RS) and several commercial cultivars in several field environments and under controlled greenhouse or poly-house conditions.

Two concurrent studies were conducted in 2001 - 2002 to compare vegetative stolon propagation of ASR368 F1 and F2 plants with that of several different creeping bentgrass commercial cultivars. Experiment I was conducted in a growth chamber in Fayette County, Kentucky and Experiment II was conducted in a poly-house in Marion County, Oregon.

Additional studies were performed during 2002 - 2003 to assess the ability of ASR368 F1 and F2 progeny and commercial cultivars to vegetatively establish under field conditions in Marion County, Oregon; Union County, Ohio; Baldwin County, Alabama and in the greenhouse in Fayette County, Kentucky. At each site the study was performed under irrigated and non-irrigated conditions. These trials were performed under USDA Notification Number 02-193-04n.

B.1. Growth chamber and poly-house vegetative establishment studies (2001 - 2002)

B.1.a. Experimental methods

<u>Experiment I – Kentucky Growth Chamber</u>

Six genotypes were evaluated in this study: ASR368 R1 (RR) and ASR368 (RS) and the commercial cultivars Penn A-4, Penncross, Crenshaw, and SR-1020. Six plants of each genotype were grown in pots in a poly-house in Marion County, Oregon and then shipped to Fayette County, Kentucky where they were acclimated in a greenhouse prior to stolon harvest for use in the study. Viable stolons were harvested from each pot based on a visual assessment of good health and the presence of two nodes within a length of 2.5 cm. Only nodes that had not yet initiated tiller production were chosen, and each stolon fragment contained exactly two nodes. Four stolon fragments (eight nodes) were clipped from each pot and trimmed to the correct length (approximately 2.5 cm) with scissors. All extraneous tissues were removed. Fragments were placed in Petri dishes (100 x 15 mm), which had been filled with approximately 50 g of soil. Stolons were pressed lightly by hand to ensure good stolon/soil contact. Dishes were then irrigated to field capacity using a wash bottle to gently apply water and not disturb stolon placement. The

experiment was initiated on December 12, 19, and 28, 2001, constituting three complete runs of the experiment, each consisting of seven days.

Dishes with stolons were placed uncovered in a plant growth chamber (Conviron Model E7, Controlled Environments, Ltd., Winnipeg, Manitoba, Canada). The environment within the chamber simulated spring/fall conditions in the transitional climatic zone. Total day length was 14 hours. Daytime high temperatures were 23°C and nighttime low temperatures were 10°C. Relative humidity was maintained as high as possible (always > 95%) to retard drying of soil and stolons in dishes. Dishes were irrigated daily to field capacity with a wash bottle. Dishes remained in the chamber for seven days during each run. At the end of the seventh day, each individual node was evaluated for initiation of new tillers.

The experimental design was completely randomized with six replications. Each Petri dish represented one replication. The stolons within a single dish were harvested from one pot or plant. Tiller counts per dish were recorded (maximum = 8, minimum = 0) at the completion of each seven-day run per planting date.

Experiment II: Oregon poly-house

Four genotypes were evaluated in this study: two independent ASR368 F2 progeny populations, ASR368a and ASR368b, and the non-transgenic commercial cultivars Penneagle and Backspin. Each ASR368 F2 population had a different non-transgenic maternal parent that had been pollinated by a random population of ASR368 F1 (Figure V.14). ASR368 progeny were each hemizygous for the cp4 epsps transgene. Each population or cultivar was represented by 50 plants.

Plants were maintained in a poly-house environment in Marion County, Oregon prior to stolon harvest. Stolons were selected and harvested at random from the population of respective plants on December 11, 2001, January 3 and February 7, 2002. The harvested stolons contained the terminal bud through the second node. The terminal bud with the first node subtending the apex was removed with a scissors from each stolon fragment and discarded. Each stolon fragment was trimmed with a scissors to 0.65 cm above and below the second node. The stolon containing the second node was then placed directly into 14 cm diameter pots containing a washed sand media that had been leveled, firmed and placed at a 10 cm depth. Each stolon fragment was planted in a vertical/upright position with the node just below the sand surface.

The experimental design was a randomized complete block with three replications at each planting date. Each experimental plot consisted of three pots containing 20 nodes each for a total of 60 nodes. All nodes were planted in sequence one replication at a time. All containers were placed on four thermostatically controlled heating mats in an enclosed poly-house. The media temperature was maintained at 16° C and monitored with soil thermometers. Each container received 20 to 25 mm of water daily via a gentle water curtain to prevent moisture stress. Day length was natural. Each of the three replications

of the study was evaluated at seven days after planting for number of nodes producing tillers (maximum = 60, minimum = 0).

Statistical analysis

Data from both experiments were transformed to percent of nodes producing at least one tiller at the end of the growth periods. Production of multiple tillers from single nodes was rare and not evaluated. Statistical analyses were conducted using PROC GLM of SAS (SAS Institute, Cary, NC). Means were separated by F-protected LSD tests (α = 0.05) after determining replication x genotype interactions did not exist. Orthogonal comparisons were used to compare ASR368 populations with non-transgenic populations in each experiment.

B.1.b. Results for experiments I and II

Nodes from ASR368 R1 RR progeny produced significantly more tillers than ASR368 RS progeny within seven days in Experiment I. However, the percentage of nodes producing tillers among ASR368 RR progeny was not significantly different from three of the four commercial creeping bentgrass cultivars including Penn A-4, Penncross and SR1020. The statistical comparisons for all three experimental runs combined are presented in Table VI.B.1.

The statistical comparisons for all three combined experimental runs of Experiment II are presented in Table VI.B.2. No significant differences (α = 0.05) for the percentage of nodes producing tillers were noted between the ASR368 genotypes or commercial cultivars tested.

Table VI.B.1. Comparison of the mean percent of nodes producing tillers of ASR368 RR and RS genotypes and four creeping bentgrass commercial cultivars after a seven-day growth period during Experiment I.

Genotype	Mean % of nodes producing tillers[1]
ASR368 (RR)	57.6a
Penn A-4	51.4ab
Penncross	44.4abc
SR-1020	41.7abc
ASR368 (RS)	37.5bc
Crenshaw	31.9c
LSD (α =0.05)	16.3

[1] Means followed by the same letter are not significantly different according to LSD (α = 0.05).

Table VI.B.2. Comparison of the mean percent of nodes producing tillers of two F2 ASR368 RR populations and two creeping bentgrass commercial cultivars after a seven-day growth period during Experiment II.

Genotype	Mean % of nodes producing tillers[1]
Backspin	55.7a
ASR368-A	54.3a
Penneagle	50.9a
ASR368-B	48.1a
LSD (α =0.05)	21.3

[1] Means followed by the same letter are not significantly different according to LSD (α = 0.05).

B.2. Vegetative establishment studies (2002 - 2003)

Field studies were established in the fall of 2002 in Marion County, Oregon; Union County, Ohio and Baldwin County, Alabama. An additional greenhouse study was also performed in Fayette County, Kentucky. The objectives of these studies were to: (1) determine if there was a difference in the ability of ASR368 to establish vegetatively compared to an elite parent plant (EPP) population and commercial creeping bentgrass cultivars under irrigated and non-irrigated field conditions and (2) supplement the results of the Kentucky greenhouse study performed in 2001, i.e. Experiment I. ASR368 F1 and F2 progeny populations and an Elite Parent Plant breeding line population plus three commercial creeping bentgrass cultivars were evaluated at each of the four locations as follows:

Backspin Alabama and Oregon
Crenshaw Alabama, Kentucky, Ohio and Oregon
Penn A-4 Alabama, Kentucky, Ohio and Oregon
Penncross Kentucky and Ohio

B.2.a. Experimental methods

<u>Plant propagation and establishment</u>

In the fall of 2001 plant material of each of the creeping bentgrass genotypes was potted and maintained in a poly-house in Marion County, Oregon. Prior to planting the Ohio and Kentucky trials in 2002, potted plants of each genotype population were sent to the Union County, Ohio location where they were maintained in a greenhouse for subsequent harvest and planting of both the Ohio and Kentucky sites.

The Oregon site maintained plant material for both the Oregon and Alabama sites. Excised stolon nodes were harvested late in the afternoon from each of the populations in Oregon for shipping via overnight early morning parcel to the Baldwin County, Alabama location for immediate planting upon receipt. Harvested stolon nodes were placed on saturated blotter paper within a Petri dish within another sealed bag to prevent

desiccation prior to shipping and planting. The second node subtending the stolon apex with ½ cm of internode on either side was cut from the plants of each population for planting at each site.

Field and greenhouse planting

Prior to planting at the Alabama, Ohio and Oregon sites, on October 11, 7 and 8, 2002, respectively, the vegetation from a 45 cm x 45 cm square in an established turf area was removed. The soil was loosened to a ½" depth to simulate a seedbed. Within this area a 30 cm x 30 cm plot was delineated into which 30 stolon nodes were introduced. The nodes were spread so that they did not overlap and were pressed uniformly into the soil surface with a board for good soil contact. The plots were arranged in a randomized complete block design with three replications per genotype per irrigation regime.

The experiment was established in the Fayette County, Kentucky greenhouse on December 10, 2002 in a similar manner but using 6" pots as individual plots.

Irrigation

Every plot was irrigated with 1 cm of water on the day of planting. Thereafter the 'irrigated' plots were irrigated daily with 0.5 cm of water. Irrigated treatments were designed to mimic a golf course where irrigation might be applied each day or where consistent precipitation events are provided each day. The 'non-irrigated' plots received only natural precipitation or 0.5 cm per week total, which was intended to mimic the conditions found in unmanaged ecosystems at each location.

Data collected

The number of nodes with viable shoots in each plot was counted a minimum of two times each week for up to five weeks per location. Based on the four locations and two irrigation regimes provided at each location, potential for vegetative establishment of transgenic ASR368 (two generations) and non-transgenic creeping bentgrasses were compared in eight different environments during this study.

Statistical analysis

Data from each location was analyzed using PROC GLM of SAS (SAS Institute, Cary, NC). Means were separated by F-protected LSD tests ($\alpha = 0.05$).

B.2.b. Results

Irrigated trials

The number of ASR368 F1 and F2 (RR) nodes producing viable tillers under irrigated conditions was not significantly different from the commercial cultivars on any

observation date in either Baldwin County, Alabama or Fayette County, Kentucky (Tables VI.B.3 and VI.B.4).

Table VI.B.3. Number of nodes out of 30 producing viable tillers under irrigated conditions among six genotype populations planted in Baldwin County, Alabama in October 2002.

Genotype population	N	Observation date (2002)									
		10/14	10/16	10/18	10/21	10/23	10/25	10/28	10/30	11/1	11/4
		Number of nodes									
Backspin	3	11.0a	19.0a	18.7a	20.0a	19.0a	20.7a	21.7a	23.3a	23.0a	21.3a
Crenshaw	3	11.7a	16.7a	16.0a	16.7a	16.0a	18.0a	22.3a	22.0a	21.3a	20.0a
EPP	3	10.7a	15.0a	15.7a	17.0a	19.0a	20.3a	22.7a	24.0a	21.7a	20.7a
Penn A-4	3	12.7a	17.3a	17.7a	17.3a	20.0a	22.0a	25.3a	25.7a	24.3a	23.7a
ASR368 F1	3	10.0a	16.0a	15.7a	17.3a	17.0a	17.7a	20.3a	20.7a	20.3a	19.7a
ASR368 F2	3	7.0a	13.7a	13.3a	15.3a	20.3a	20.3a	21.7a	23.3a	23.0a	23.0a
LSD ($\alpha = 0.05$)[1]		7.70	7.12	6.78	6.53	6.67	4.82	5.45	4.66	4.59	5.29
Pr > F		0.681	0.653	0.613	0.741	0.668	0.380	0.497	0.325	0.478	0.497

[1] Means with the same letter during each observation date are not significantly different, $\alpha = 0.05$.

Table VI.B.4. Number of nodes out of 30 producing viable tillers under irrigated conditions among six genotype populations planted in Fayette County, Kentucky in December 2002.

Genotype population	N	Observation date (2002)						2003
		12/16	12/18	12/20	12/23	12/25	12/27	1/3
		Number of nodes						
Penncross	3	20.7a	24.7a	26.0a	26.7a	27.7a	25.3a	26.3a
Crenshaw	3	20.3a	26.3a	29.0a	29.0a	29.3a	28.0a	32.7a
EPP	3	20.3a	21.3a	24.7a	25.7a	25.7a	28.3a	28.0a
Penn A-4	3	20.0a	24.0a	27.0a	27.7a	27.7a	26.3a	27.7a
ASR368 F1	3	18.3a	21.3a	26.3a	26.7a	28.3a	26.0a	30.7a
ASR368 F2	3	17.0a	23.3a	27.0a	27.3a	27.7a	26.0a	31.3a
LSD ($\alpha = 0.05$)[1]		8.57	7.47	4.96	5.07	4.17	4.71	7.71
Pr > F		0.919	0.668	0.578	0.790	0.579	0.682	0.474

[1] Means with the same letter during each observation date are not significantly different, $\alpha = 0.05$.

At the Union County, Ohio location, the ASR368 F2 produced significantly fewer tillers than Penn A-4 and EPP populations by October 15, 2002 but was not significantly different from any non-transgenic populations on the remaining observation dates. The ASR368 F1 had significantly more stolon nodes than Crenshaw on October 18, 2002 but was not significantly different from the other commercial cultivars or the EPP population on that and all other observation dates in Union County, Ohio (Table VI.B.5).

Table VI.B.5. Number of nodes out of 30 producing viable tillers under irrigated conditions among six genotype populations planted in Union County, Ohio in October 2002.

Genotype population		Observation date (2002)					
	N	10/15	10/18	10/22	10/25	10/30	11/5
		Number of nodes					
Penncross	3	7.7bcd	9.7ab	10.7b	10.7b	12.7ab	12.0ab
Crenshaw	3	7.0cd	7.7b	9.0b	9.3b	9.7b	9.7b
EPP	3	11.0a	12.7a	14.7a	14.7a	14.7a	14.7a
Penn A-4	3	10.0ab	11.7a	12.7ab	12.7ab	13.0ab	13.0ab
ASR368 F1	3	9.3abc	11.3a	11.3ab	11.3ab	12.0ab	12.0ab
ASR368 F2	3	5.3d	9.7ab	11.7ab	12.0ab	13.0ab	12.7ab
LSD ($\alpha = 0.05$)[1]		2.77	3.63	3.68	3.45	4.10	4.42
Pr > F		0.010	0.109	0.088	0.080	0.247	0.319

[1] Means with the same letter during each observation date are not significantly different, $\alpha = 0.05$.

In Marion County, Oregon, Backspin had significantly more nodes than the other genotype populations including, ASR368 F1 and F2, from October 21 through October 31, 2002. ASR368 F1 had significantly more nodes than the EPPs on October 21 and 25, 2002 and ASR368 F2 had significantly fewer nodes than the EPPs on October 14, 2002 (Table VI.B.6). However, these differences were not consistent and the ASR368 F1 and F2 fell within the range of the commercial cultivars on all dates.

Table VI.B.6. Number of nodes out of 30 producing viable tillers under irrigated conditions among six genotype populations planted in Marion County, Oregon in October 2002.

Genotype population	Observation date (2002)							
	N	10/11	10/14	10/18	10/21	10/25	10/28	10/31
	Number of nodes							
Backspin	3	0.0a	5.3a	16.7a	22.7a	22.3a	22.7a	22.7a
Crenshaw	3	0.0a	1.7c	8.0b	13.3cb	13.3bc	13.3b	13.3b
EPP	3	0.0a	4.7a	9.3b	11.3c	11.3c	11.3b	11.3b
Penn A-4	3	0.0a	2.3bc	9.0b	12.3bc	13.0bc	12.7b	12.7b
ASR368 F1	3	0.0a	3.7ab	12.3ab	15.7b	16.0b	15.7b	15.7b
ASR368 F2	3	0.0a	2.0bc	8.0b	13.3bc	13.3bc	12.7b	12.7b
LSD ($\alpha = 0.05$)[1]		0.00	1.96	4.79	4.24	3.90	4.55	4.55
Pr > F		na	0.008	0.015	0.002	0.001	0.003	0.003

[1] Means with the same letter during each observation date are not significantly different, $\alpha = 0.05$.
na not available, due to 0.0 counts neither a Pr > F or LSD value could be calculated

Non-irrigated trials

The number of ASR368 F1 and F2 nodes producing viable tillers under non-irrigated conditions was not significantly different from the non-transgenic creeping bentgrass populations on all evaluation dates at all locations other than two instances (Tables VI.B.7 – VI.B.10). On October 18, 2002, in Baldwin County, Alabama ASR368 F1 had significantly more nodes than the EPPs and on October 30, 2002 ASR368 F2 had significantly fewer nodes than Crenshaw. It is clear from the results at the Kentucky, Ohio and Oregon locations that irrigation is necessary to supplement precipitation for the successful establishment of creeping bentgrass stolon nodes at these locations.

Table VI.B.7. Number of nodes out of 30 producing viable tillers under non-irrigated conditions among six genotype populations planted in Baldwin County, Alabama in October 2002.

Genotype population		Observation date (2002)									
	N	10/14	10/16	10/18	10/21	10/23	10/25	10/28	10/30	11/1	11/4
		Number of nodes									
Backspin	3	9.0a	17.3a	17.0a	13.0a	14.3a	12.3a	14.7a	13.3a	12.7a	11.7a
Crenshaw	3	6.3ab	16.7a	16.7ab	13.0a	13.0a	13.3a	15.0a	14.3a	14.0a	14.3a
EPP	3	5.3b	11.3b	10.7c	12.3a	12.7a	12.0a	15.7a	17.0a	16.0a	15.7a
Penn A-4	3	6.3ab	14.0ab	12.7bc	10.0a	9.3a	9.3a	12.7a	12.0a	11.7a	10.7a
ASR368 F1	3	6.0ab	14.3ab	16.3ab	15.0a	12.0a	12.3a	13.0a	13.0a	12.3a	13.7a
ASR368 F2	3	8.3ab	14.3ab	14.7abc	13.0a	13.3a	12.7a	14.0a	12.7a	12.0a	12.0a
LSD ($\alpha = 0.05$)[1]		3.61	4.96	4.03	6.76	8.66	8.72	11.58	11.94	11.5	11.27
Pr > F		0.253	0.191	0.031	0.726	0.8472	0.931	0.990	0.945	0.956	0.917

[1] Means with the same letter during each observation date are not significantly different, $\alpha = 0.05$.

Table VI.B.8. Number of nodes out of 30 producing viable tillers under non-irrigated conditions among six genotype populations planted in Fayette County, Kentucky in December 2002.

Genotype population		Observation date (2002)						2003
	N	12/16	12/18	12/20	12/23	12/25	12/27	1/3
		Number of nodes						
Penncross	3	11.0a	5.0a	1.7a	0.7a	0.0a	0.0a	0.0a
Crenshaw	3	14.0a	10.0a	4.0a	2.0a	1.0a	0.0a	0.0a
EPP	3	13.0a	12.3a	7.7a	4.3a	2.7a	0.0a	0.0a
Penn A-4	3	7.0a	5.0a	1.0a	0.0a	0.0a	0.0a	0.0a
ASR368 F1	3	17.0a	11.0a	3.0a	2.0a	1.7a	0.0a	0.0a
ASR368 F2	3	11.0a	6.0a	2.0a	1.3a	0.7a	0.0a	0.0a
LSD ($\alpha = 0.05$)[1]		11.80	11.65	9.32	4.93	3.08	0.00	0.00
Pr > F		0.586	0.603	0.674	0.525	0.425	na	na

[1] Means with the same letter during each observation date are not significantly different, $\alpha = 0.05$.

Table VI.B.9. Number of nodes out of 30 producing viable tillers under non-irrigated conditions among six genotype populations planted in Union County, Ohio in October 2002.

Genotype population		Observation date (2002)					
	N	10/15	10/18	10/22	10/25	10/30	11/5
		Number of nodes					
Penncross	3	0.0a	0.0a	0.0a	0.0a	0.0b	0.0a
Crenshaw	3	1.0a	1.3a	1.3a	1.3a	1.0a	0.0a
EPP	3	0.0a	0.0a	0.0a	0.0a	0.3ab	0.3a
Penn A-4	3	0.3a	0.3a	0.7a	0.7a	0.3ab	0.3a
ASR368 F1	3	1.0a	0.7a	0.7a	0.7a	0.3ab	0.3a
ASR368 F2	3	0.0a	0.3a	0.3a	0.3a	0.0b	0.0a
LSD ($\alpha = 0.05$)[1]		1.65	1.47	1.49	1.49	1.00	0.81
Pr > F		0.529	0.395	0.396	0.396	0.326	0.770

[1] Means with the same letter during each observation date are not significantly different, $\alpha = 0.05$.

Table VI.B.10. Number of nodes out of 30 producing viable tillers under non-irrigated conditions among six genotype populations planted in Marion County, Oregon in October 2002.

Genotype population		Observation date (2002)						
	N	10/11	10/14	10/18	10/21	10/25	10/28	10/31
		Number of nodes						
Backspin	3	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Crenshaw	3	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
EPP	3	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Penn A-4	3	0.0a	0.0a	0.0a	0.3a	0.3a	0.0a	0.0a
ASR368 F1	3	0.0a	0.0a	0.3a	0.0a	0.0a	0.0a	0.0a
ASR368 F2	3	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
LSD ($\alpha = 0.05$)[1]		0.00	0.00	0.43	0.43	0.43	0.00	0.00
Pr > F		na	na	0.465	0.465	0.465	na	na

[1] Means with the same letter during each observation date are not significantly different, $\alpha = 0.05$.

B.3. Conclusions for vegetative establishment studies

The results of the vegetative establishment experiments described above demonstrate that Roundup tolerance imparted by plant transformation had neither a positive or negative effect on the production of tillers from the stolon nodes of two progeny populations of ASR368. These results are consistent with the data and information developed by Monsanto to demonstrate that the vegetative growth of genetically modified glyphosate tolerant soybean, canola, corn, cotton and sugarbeet was not different from non-transgenic cultivars of the same species (Monsanto, 1993, 1995, 1997, 1998, 2000, 2002). This information formed the basis for their deregulation under the Plant Pest Act by the United States Department of Agriculture

It is important to note that even though the environmental and edaphic conditions in Experiments I and II (SectionVI.B.1.a) were different, the maximum mean percentage of nodes producing tillers was essentially the same (maximum mean percentage; Exp. I = 57.6%, Exp. II = 55.7%). Statistical differences did occur among the RR and RS ASR368 genotypes in Experiment I (but not in Experiment II). However, both Holt and Payne (1951) and Cattani (1999) also reported differences in tillers and stolons among different creeping bentgrass genotypes grown under the same conditions.

The results of the 2002 field and greenhouse studies lend further support to the conclusion that ASR368 plants are not different from commercial or non-transgenic creeping bentgrass cultivars in their ability to produce new tillers from viable stolon nodes. This supports a conclusion of no contribution to increased weed potential based on the vegetative establishment characteristics of ASR368 compared to related EPP plants or commercial creeping bentgrass cultivars that are representative of *A. stolonifera.*

C. Relative Growth

Turfgrasses are perennial plants not because individual shoots survive indefinitely, but because the plant community is dynamic (Turgeon, 2002). During vegetative growth and development, there is no basic alteration in the structural design of the turfgrass plant. Organs originate in a repetitious well-defined manner following a typical sigmoidal curve. The size of the plant and the number of plant organs, e.g., roots, tillers, leaves, etc. typically does not change (Turgeon, 2002).

Tillers, the basic unit of the plant, arise from axillary buds. They are most active in the late summer to early autumn and may function vegetatively or reproductively according to environmental stimuli (Turgeon, 2002). Tillers enable the in-place expansion of the plant and usually live for not more than one year and frequently less (Turgeon, 1985). Jonsdottir (1991) reported that creeping bentgrass tillers had a very short life span with a half-life of two to seven months. Mortality of a tiller typically occurs after flowering, which is usually followed by replacement with a new tiller. The number of leaves per tiller remains fairly constant and the rate of new leaf emergence is approximately the same as the rate of senescence. A turfgrass plant is considered mature when tillers are receptive to floral induction (Turgeon, 1985). However, grasses maintained as turfgrasses may not go through a reproductive phase because the mowing height and regularity of mowing, particularly on golf courses, precludes flowering (Lush 1988; Johnson and Riordan, 1999).

Stolons lead to the production of independently rooted daughter plants at stolon nodes (Cattani and Struik, 2001). Attached stolons generally develop as competition within the plant necessitates better light for growth (Cattani and Struik, 2001). *A. stolonifera* can exhibit substantial stolon growth (Eriksson, 1989; Watschke, 1995) under favorable environmental conditions, i.e., good mineral nutrition and water availability (Hunt *et al*., 1987).

Golf courses manage creeping bentgrass with frequent close mowing, aggressive cultivation and sufficient agronomic inputs to achieve the highest vertical tiller density (shoots per unit area) and shortest internode length possible. Large numbers of individual plants that contribute to visual and physical uniformity across the entire sward are favored over small numbers of large clonal (stolon generated) colonies under these conditions. Seed producers prefer individual plants to enhance the potential for reproductive tillering. Consequently, seed is planted in rows and the top-growth is removed after harvest to encourage lateral stolon growth.

In this section, we examine the relative growth of ASR368 compared to non-transgenic creeping bentgrass. Increased growth, either when established in bare soil or in competition with other turfgrasses could increase the plant pest potential of ASR368. Studies were performed across multiple locations representing the northern or cool, southern or warm and transition climate zones of turfgrass adaptation in 2000, 2001, 2002 and 2003 (USDA # 99-203-04n, 00-159-02n, 00-224-01n, 00-201-03n, 01-151-02n, 01-177-02n and 02-193-04n). Seasonal variation over the duration of the studies

contributed to additional stresses within each study location. The studies performed, identity of ASR368 test material, control plants and cultivars, locations and study duration are provided in Tables VI.C.1 and VI.C.2.

Table VI.C.1. ASR368, B99061R, and commercial bentgrass cultivars evaluated for relative growth and competitive ability in 2000 – 2003.

Locations County, State	Climate Zone	Planting Date (m/d/y)	Competitive Turf[1]	ASR368 Generation	Bentgrass Cultivars Evaluated[2]	Study Duration (mos)[3]
Clinton, IL	Transition	7/26/00	Bare soil	R0	B99061R, CR, PE, P4	13
Ottawa, MI	Cool	7/27/00	Bare soil	R0	B99061R, CR, PE, P4	13
Union, OH	Cool	6/23/00	Bare soil	R0	B99061R, CR, PE, P4	15
Marion, OR	Cool	6/28/00	Bare soil	R0	B99061R, CR, PE, P4	14
Middlesex, NJ	Transition	7/14/00	KB	R0	B99061R, CR, PE, P4	25
Union, OH	Cool	6/23/00	KB	R0	B99061R, CR, PE, P4	14
Franklin, OH	Cool	10/31/00	PR	F1	B99061R, BS, CR, PE, P4, HB, ST, SR	24
Marion, OR	Cool	6/20/00	KB/PR	R0	B99061R, CR, PE, P4	32
Baldwin, AL[4]	Warm	11/2/00	BG	F1	B99061R, BS, CR, PE, P4, HB, ST, SR	9
Baldwin, AL[5]	Warm	11/2/00	SA	F1	B99061R, BS, CR, PE, P4, HB, ST, SR	9
Baldwin, AL[4]	Warm	11/2/01	BG	F1	B99061R, BA, CR, PE, P4, HB, ST,SEA	9
Baldwin, AL[5]	Warm	11/2/01	SA	F1	B99061R, BA, CR, PE, P4, HB, ST,SEA	9
Ingham, MI	Cool	3/31/01	Bare soil	F1	B99061R, BS, CR, PE, P4, HB, ST, SR	6
Ingham, MI	Cool	2/2/02	Bare soil	F1	B99061R, BS, CR, PE, P4, HB, ST, SR	4

[1] Mature turf varieties into which ASR368 and other test genotypes were planted: KB = Kentucky bluegrass, PR = perennial ryegrass, BG = bermudagrass, SA = St. Augustinegrass, KB/PR = a uniform mixture of Kentucky bluegrass and perennial ryegrass

[2] Bentgrass cultivars tested: BA = Bardot colonial bentgrass (*Agrostis capillaris*), BS = Backspin, CR = Crenshaw, PE = Penncross, P4 = Penn A-4, HB = Highland dryland bentgrass (*Agrostis castellana*), ST = Streaker redtop (*Agrostis gigantea*) and SR = SR7100 colonial bentgrass (*Agrostis capillaris*)

[3] Months post-planting

[4] ASR368, B99061R and bentgrass cultivars were planted into direct sun

[5] ASR368, B99061R and bentgrass cultivars were planted in the shade to assess shade tolerance

Table VI.C.2. Months in which observations were made of relative plant growth and competitive ability at each location during 2000 to 2003.

Location	2000						2001												2002												2003	
	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
Clinton, IL		*	*	*	*					*	*	*	*	*																		
Ottawa, MI		*	*	*	*			*	*	*	*	*	*	*																		
Union, OH				*							*	*	*	*	*																	
Marion, OR		*	*	*				*	*	*	*	*	*	*																		
Franklin, OH										*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*				
Union, OH	*	*	*		*				*	*	*	*	*	*																		
Middlesex, NJ		*	*	*							*	*	*													*						
Marion, OR		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*				*
Baldwin, AL (sun)									*	*	*	*	*	*																		
Baldwin, AL (shade)									*	*	*	*	*	*																		
Baldwin, AL (sun)																						*	*	*	*	*						
Baldwin, AL (shade)																						*	*	*	*	*						
Ingham, MI (shade)												*	*		*																	
Ingham, MI (shade)																				*	*	*	*									

C.1. Experiment I - relative growth in bare soil without mowing

Studies were conducted during 2000 – 2001 to compare the relative growth rates of un-mowed ASR368 R0 generation plants, B99061R and three commercial creeping bentgrass cultivars (Crenshaw, Penncross, and Penn A-4) in non-competitive, bare soil environments.

The experiment was conducted at four locations representing a range of cool season and transition zone environments where creeping bentgrass is grown: Clinton County, Illinois; Marion County, Oregon; Union County, Ohio and Ottawa County, Michigan. These field releases were conducted under USDA # 00-159-02n and 01-151-02n.

C.1.a. Experimental methods

Site establishment and maintenance

The starting material for each creeping bentgrass genotype consisted of stolon nodes grown in Jiffy® Pellets (42 mm) in a poly-house in Marion County, Oregon during spring 2000. Each field location was established with three replicated 3' x 5' plots per treatment

arranged in a randomized complete block design. Each plot was populated with three plants of a single genotype. Vegetative plugs of each entry were planted at a depth of two to four inches and were separated from the adjoining plot by a buffer of at least five feet.

The plants at each location were managed with standard agronomic practices to maintain plant growth. Irrigation and insecticide and fungicide applications were applied as needed to maintain the integrity of the plots and fertilizer was applied to avoid nutrient deficiency symptoms. Although the plants were not mowed, individual seed heads were removed manually as necessary to preclude pollen development.

Data collection

Data was collected on a near monthly basis between the summer of 2000 and the fall of 2001 (Table VI.C.2). Early season evaluations consisted of measuring the minimum and maximum stolon length for each of three plants within each plot. As the plants matured, treatment evaluations were made by measuring the percentage ground cover within each 3 x 5' plot and shoot density. A 1 to 9 rating scale, where 9 was the greatest number of shoots per unit area, was used to assess shoot density. This change in data collection facilitated a more rapid and direct assessment of development since the plants within each plot had grown into one another and precluded additional stolon length measurements.

Statistical analysis

Analysis of variance was used to determine differences in growth among the bentgrass lines. Means were compared using Fisher's LSD ($\alpha = 0.05$). Fisher's LSD was chosen because it has the highest power of the pair-wise separation procedures

C.1.b. Results

Ottawa County, Michigan

The Ottawa County, Michigan location was planted on July 27, 2000 and represents a cool season climate. Early season observations of stolon length were taken from August 27, 2000 through March 28, 2001. Each mean represents the average of two observations each on nine plants (i.e., three plots, three plants per plot). Observations taken after March 28, 2001 reflect the percent ground cover in the plots and relative shoot density (shoots / unit area). Values for shoot density were recorded on a 1 to 9 scale with 1 being low and 9 being high.

Stolon Length:

There were no differences in stolon length, percentage plot cover or shoot density observed between ASR368, B99061R and the commercial creeping bentgrass cultivars according to Fisher's LSD ($\alpha = 0.05$) over the duration of the trial.

Therefore, with respect to stolon growth, percentage ground cover and shoot density, ASR368 was not different from B99061R or the commercial creeping bentgrass cultivars evaluated at this location between August 2000 and October 2001 (Tables VI.C.3 and VI.C.4).

Table VI.C.3. Plant growth as measured by stolon length (cm) of ASR368 R0, B99061R and three commercial cultivars in Ottawa County, Michigan from August, 2000 to March, 2001.

Genotype	2000												2001					
	August			September			October			November			February			March		
	centimeters																	
	Mx[1]	X[2]	Mn[3]	Mx	X	Mn	Mx	X	Mn	Mx	X	Mn	Mx	X	Mn	Mx	X	Mn
Crenshaw	70	42.3	28	89	53.0	33	93	59.0	37	106	61.3	41	90	57.0	35	102	61.3	37
Penn A-4	55	34.7	17	64	51.3	28	94	63.7	40	96	65.0	38	88	58.7	36	92	63.3	38
Penncross	77	46.7	35	98	61.0	45	102	65.0	43	108	66.3	40	96	63.0	40	98	67.0	37
B99061R	56	40.3	25	84	54.7	40	81	63.3	41	98	64.3	38	97	57.3	38	90	62.3	47
ASR368 R0	64	42.7	28	75	57.7	36	93	67.0	43	97	69.0	44	90	64.3	43	90	67.7	40
LSD (0.05)		NS[4]			NS			NS			NS			NS			NS	
Trt. Prob (F)		0.17			0.33			0.60			0.56			0.64			0.69	
CV		12.6			10.3			9.4			8.3			12			10.1	

[1] Mx = maximum.
[2] Means (X) were calculated from the maximum (Mx) and minimum (Mn) values from each of three plants per replicate (n=9).
[3] Mn = minimum.
[4] NS = not significant according to Fisher's LSD ($\alpha = 0.05$).

Table VI.C.4. Plant growth as measured by percentage ground cover and shoot density[1] of ASR368 R0, B99061R and three commercial cultivars in Ottawa County, Michigan from April, 2001 to July, 2001.

	2001							
Genotype	April		May		June		July	
	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density
Crenshaw	48.3	8.0	58.3	8.7	66.7	8.0	78.3	9.0
Penn A-4	51.7	7.7	65.0	8.3	75.0	8.7	85.0	9.0
Penncross	66.7	8.0	78.3	8.3	83.3	7.7	95.0	8.3
B99061R	45.0	6.3	50.0	7.7	60.0	8.0	78.3	8.7
ASR368 R0	50.0	7.3	66.7	8.3	76.7	8.3	85.7	8.7
LSD (0.05)	NS[2]	NS	NS	NS	NS	NS	NS	NS
Trtmnt Prob (F)	0.204	0.217	0.125	0.265	0.281	0.211	0.395	0.232
CV	20.1	11.9	18.1	6	17.6	5.9	13	4.2

[1] Shoot density (shoots per unit area) was recorded on a 1-9 scale with 1 being low and 9 being high
[2] NS = not significant according to Fisher's LSD ($\alpha = 0.05$).

Clinton County, Illinois

The Clinton County, Illinois location represents a transition zone climate for cool and warm season grass adaptation. The location was planted on July 26, 2000. Early season observations of stolon length were taken from August 30, 2000 through April 15, 2001. Observations taken after April 15, 2001 reflect percent ground cover and relative shoot density (shoots / unit area). Values for percentage ground cover and shoot density were recorded in the same manner as described previously for Ottawa County, Michigan.

Throughout two seasons of growth in Clinton County, Illinois, the stolon length, percentage ground cover and shoot density of ASR368 R0 plants were not significantly different from either B99061R or Crenshaw. ASR368 R0 was significantly larger than Penn A-4 in September 2000, and April and June 2001 and significantly smaller than Penncross in July and August 2001 (Table VI.C.5 and Table VI.C.6). No significant differences between ASR368 and the non-transgenic creeping bentgrasses were detected on any other dates.

Table VI.C.5. Comparative growth as measured by stolon length (cm) of ASR368 R0, B99061R and three commercial cultivars in Clinton County, Illinois from August 2000 to April 2001.

	2000												2001		
Genotype	**August**			**September**			**October**			**November**			**April**		
	centimeters														
	Mx[1]	Mean[2]	Mn[3]	Mx	Mean[5]	Mn	Mx	Mean	Mn	Mx	Mean	Mn	Mx	Mean[5]	Mn
Crenshaw	56	29.7	16	74	39.0a	19	76	39.7	25	80	43	30	68	35.3a	18
Penn A-4	46	24.7	12	41	22.3b	7	46	30.3	15	60	39.7	20	39	24.7b	13
Penncross	52	33.7	22	62	42.0a	29	60	44	30	70	50	25	57	42.0a	30
B99061R	58	30	16	53	38.0a	19	50	36.7	20	55	37.7	25	49	35.0a	24
ASR368 R0	49	30.3	18	51	35.0a	20	50	35.3	20	55	37.3	15	52	33.7a	26
LSD (0.05)		NS[4]			10.9			NS			NS			8.6	
Trt Prob (F)		0.215			0.023			0.235			0.41			0.019	
CV		13.9			16.4			17.9			20.6			13.3	

[1] Mx = maximum.
[2] Means were calculated from the maximum (Mx) and minimum (Mn) values from each of three plants per replicate (n=9).
[3] Mn = minimum.
[4] NS = not significant according to Fisher's LSD ($\alpha = 0.05$).
[5] Means not followed by the same letter are significantly different according to Fisher's LSD ($\alpha = 0.05$).

Table VI.C.6. Comparative growth as measured by percentage ground cover and shoot density[1] of ASR368 R0, B99061R and three commercial cultivars in Clinton County, Illinois from May, 2001 to August, 2001.

	2001							
Genotype	**May**		**June**		**July**		**August**	
	% Cover	Density	% Cover[3]	Density	% Cover[3]	Density	% Cover[3]	Density
Crenshaw	58.3	7.0	71.7a	6.5	58.3ab	8.0	66.7ab	7.7
Penn A-4	35.0	5.8	31.7b	6.7	40.0c	7.0	43.3c	6.8
Penncross	71.7	8.0	76.7a	6.0	66.7a	8.3	75.0a	7.7
B99061R	51.7	7.8	65.0a	8.3	60.0ab	8.3	63.3ab	7.8
ASR368 R0	53.3	6.7	55.0a	7.7	45.0bc	7.7	55.0bc	7.2
LSD (0.05)	NS[2]	NS	22.0	NS	18.4	NS	19.1	NS
Trtmnt Prob (F)	0.09	0.46	0.01	0.09	0.049	0.24	0.04	0.42
CV	25.2	21.8	19.4	13.6	18.1	9.4	16.8	9.3

[1] Shoot density (shoots per unit area) was recorded on a 1-9 scale with 1 being low and 9 being high.
[2] NS = not significant according to Fisher's LSD ($\alpha = 0.05$).
[3] Means not followed by the same letter are significantly different according to Fisher's LSD ($\alpha = 0.05$).

Marion County, Oregon

The Marion County, Oregon location was planted on June 28, 2000. Oregon's cool season and mild climate provided for additional growth of individual plants relative to other sites. The rapid increase in plant growth permitted a measurement of stolon size only in August and November 2000. Percentage ground cover was recorded on the observation dates from September through April 2001, and from May to August 2001 both percent ground cover and shoot density were recorded. Values for shoot density were recorded as described above.

Throughout two seasons of growth in Marion County, Oregon the growth of the ASR368 R0 plants was not significantly different from B99061R and the three commercial cultivars (Tables VI.C.7 – VI.C.9).

Table VI.C.7. Comparative growth as measured by stolon length (cm) of ASR368 R0, B99061R and three commercial cultivars in Marion County, Oregon in August, 2000 and November 2000.

	2000					
Genotype	**August**			**November**		
	centimeters					
	Max[1]	Mean[2]	Min[3]	Max	Mean	Min
Crenshaw	52	39.7	24	129	103.0	52
Penn A-4	61	40.7	23	149	101.7	66
Penncross	57	49.7	38	136	111.3	84
B99061R	54	42.7	33	128	106.3	80
ASR368 R0	52	41	29	127	99.3	80
LSD (0.05)		NS[4]			NS	
Trtmnt Prob (F)		0.08			0.83	
CV		9.2			12.8	

[1] Max = maximum.
[2] Means were calculated from the maximum (Max) and minimum (Min) values from each of three plants per replicate (n=9).
[3] Min = minimum.
[4] NS = not significant according to Fisher's LSD ($\alpha = 0.05$).

Table VI.C.8. Comparative growth as measured by percentage ground cover of ASR368 R0, B99061R and three commercial cultivars in Marion County, Oregon from September 2000 to March, 2001.

Genotype	**2000**		**2001**	
	September	**October**	**February**	**March**
	% Cover	**% Cover**	**% Cover**	**% Cover**
Crenshaw	61.0	80.0	84.3	78.3
Penn A-4	63.3	76.7	88.7	82.7
Penncross	74.3	94.3	92.3	90.7
B99061R	54.3	76.7	87.0	88.3
ASR368 R0	51.7	75.0	83.7	80.0
LSD (0.05)	NS[1]	NS	NS	NS
Treatment Prob (F)	0.32	0.52	0.72	0.33
CV	21.4	18.3	9.6	9.4

[1] Shoot density observations were recorded on a 1-9 scale with 1 being low and 9 being high.
[2] NS = not significant according to Fisher's LSD ($\alpha = 0.05$).

Table VI.C.9. Comparative growth as measured by percentage ground cover and shoot density[1] of ASR368 R0, B99061R and three commercial cultivars in Marion County, Oregon from May, 2001 to August, 2001.

	2001							
Genotype	**May**		**June**		**July**		**August**	
	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density
Crenshaw	96.7	6.3	96.7	7	100	6.0bc[3]	100	6.3
Penn A-4	75.0	7	78.3	6.3	90	8.0a	91.7	6.7
Penncross	100	6.3	100	6	100	6.7bc	100	6.3
B99061R	99.3	7	99.3	7.3	100	7.7a	100	8.0
ASR368 R0	99.3	7	98.3	7.3	100	7.3ab	100	6.7
LSD (0.05)	NS[2]	NS	NS	NS	NS	0.9	NS	NS
Treatment Prob (F)	0.44	0.77	0.44	0.06	0.46	0.01	0.46	0.18
CV	19.3	14.1	16.4	8.1	7.9	6.8	6.6	10.9

[1] Shoot density observations were recorded on a 1-9 scale with 1 being low and 9 being high.
[2] NS = not significant according to Fisher's LSD ($\alpha = 0.05$).
[3] Means followed by the same letter on date are not significantly different according to Fisher's LSD ($\alpha = 0.05$).

Union County, Ohio

The Union County, Ohio location was planted on June 23, 2000 and represents a cool season climate. An observation of stolon length was taken on October 3, 2000. Due to winter conditions no observations were taken again until May 4, 2001. From this date forward observations of percent ground cover and shoot density were taken.

Throughout two seasons of growth in Union County, Ohio, the stolon length, percentage ground cover and/or shoot density of ASR368 R0 were not significantly different from that of B99061R and Penn A-4. ASR368 R0 had a significantly lower percent cover in June through September 2001 and significantly lower density in August and September 2002 than Crenshaw. ASR368 R0 also had a lower percent cover and density in August and less coverage in September 2002 than Penncross (Table VI.C.10 and Table VI.C.11).

Table VI.C.10. Comparative growth as measured by stolon length (cm) of ASR368 R0, B99061R and three commercial cultivars in Union County, Ohio in October, 2000.

	October 2000		
	centimeters		
Genotype	**Max[1]**	**Mean[2]**	**Min[3]**
Crenshaw	99	45.7	21
Penn A-4	63	38.3	14
Penncross	65	41.7	26
B99061R	65	40	19
ASR368 R0	81	43.7	19
LSD (0.05)		NS[4]	
Treatment Prob(F)		0.69	
CV		15.9	

[1] Max = maximum.
[2] Means were calculated from the maximum (Max) and minimum (Min) values from each of three plants per replicate (n=9).
[3] Min = minimum.
[4] NS = not significant according to Fisher's LSD ($\alpha = 0.05$).

Table VI.C.11. Comparative growth as measured by percentage ground cover and shoot[1] density of ASR368 R0, B99061R and three commercial cultivars in Union County, Ohio from May, 2001 to September, 2001.

	2001									
Genotype	**May**		**June**		**July**		**August**		**September**	
	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density	% Cover	Density
Crenshaw	36.7a[3]	5.3	43.3a	6.3	53.3a	6.7	36.7a	5.0a	58.3a	5.7a
Penn A-4	17.7b	3.7	13.3c	5.7	23.3bc	5.0	13.3b	1.7c	18.3b	3.3b
Penncross	37.7a	5.3	33.3ab	6.3	33.3b	6.7	36.7a	4.0ab	46.7a	4.3ab
B99061R	27.0ab	4.7	11.7c	7.0	13.3c	4.3	15.0b	2.7bc	26.7b	3.3b
ASR368 R0	26.7ab	5.0	20.0bc	7.0	23.3bc	5.3	15.0b	2.0c	26.7b	3.3b
LSD (0.05)	13.2	NS[2]	15.7	NS	19.7	NS	19.7	1.8	16	1.6
Treatment Prob (F)	0.04	0.09	0.01	0.13	0.01	0.15	0.04	0.01	0.002	0.05
CV	24.2	14.5	34.2	9.6	35.7	21.4	44.9	30.9	24.1	21.3

[1] Shoot density was recorded on a 1-9 scale with 1 being low and 9 being high.
[2] NS, not significant according to Fisher's LSD ($\alpha = 0.05$).
[3] Means followed by the same letter on date are not significantly different according to Fishers LSD ($\alpha = 0.05$)

C.1.c. Conclusions from bare soil relative growth studies

Throughout the 13 to 15 months that these studies were performed during 2000 and 2001, ASR368 R0 plants were not significantly different from B99061R and significantly different from the commercial cultivars on only a few dates. These results support the conclusion that ASR368 plants will not differ in establishment and growth rate from non-transgenic creeping bentgrass genotypes when planted into bare soil under non-competitive conditions. Furthermore, ASR368 plants show no additional growth ability related to shoot density or ability to colonize open ground compared to non-transgenic creeping bentgrass, which further supports a conclusion of no contribution to increased weed potential based on these growth characteristics.

C.2. Experiment II - Relative growth in competitive and managed turfgrass stands

A number of experiments were performed in 2000, 2001 and 2002 to evaluate the relative growth of ASR368 compared to a number of commercial cultivars in competitive managed turfgrass stands. These experiments were conducted at six locations representing several distinct environments: (1) cool season, (2) transitional climate, (3) warm season full sun, (4) warm season shade and (5) reduced irradiance (Table VI.C.1). Each of the cool and transitional climate zone studies was conducted for a minimum of two growing seasons, including the year of establishment as presented in Table VI.C.2. The trials in Alabama and Michigan were repeated in 2001 and 2002 and 2000 and 2002, respectively.

C.2.a. Relative growth of ASR368 R0 generation plants in competitive and managed cool season and transition zone turfgrass regions

C.2.a.1. Experimental methods

Field studies were initiated on June 23, 2000 in Union County, Ohio; October 31, 2000 in Franklin County, Ohio; July 14, 2000 in Middlesex County, New Jersey, and June 20, 2000 in Marion County, Oregon. The turf area (perennial ryegrass, and/or Kentucky bluegrass) to be inter-planted was maintained for uniform turf coverage and surface drainage (Table VI.C.1). Soil cores (35 mm in diameter and 60 mm deep) were removed from the original turf area on 60 – 90 cm centers. Bentgrass plugs were transplanted directly into the core holes so that the crowns of the plant were at or slightly below the soil surface to ensure firm contact between the Jiffy pot media was maintained with the field soil. The turf area was maintained under a regime appropriate for the original dominant species following an initial establishment phase to acclimate the bentgrass transplants.

The plots were irrigated as needed to prevent stress during a six-week establishment phase, and then irrigated to maintain the existing turf. Plots were mown regularly at a clipping height of 1.25 cm in Oregon, 3.75 cm in New Jersey, and 5 cm or less at both Ohio locations. Nitrogen and other nutrients were applied as needed to compensate for

visual deficiency symptoms. Insecticides and fungicides were applied as needed to control the integrity of plots following standard agronomic practices.

Bentgrass plant diameter was measured in centimeters each month of the growing season when possible. Mean plant diameter was calculated as an average of two measurements made on each tiller plot.

Statistical analysis

The study was conducted as a completely randomized design with three replications at each of four locations. Analysis of variance was used to determine differences in growth among the bentgrass lines. Means were compared using Fisher's LSD ($\alpha = 0.05$).

The data were analyzed over all locations with lines nested within plant type (control, reference, or transgenic). However, the number of reporting locations varied by month as listed in Table VI.C.2. Data collection at the Union County, Ohio and Middlesex County, New Jersey sites was restricted during several winter months (November to April) by snow cover and weather conditions. Variation from site to site necessitated a separate analysis of monthly data from each location.

C.2.a.2. Results

Middlesex County, New Jersey (transition zone climate)

Mean plant diameter measurements were made from August through October 2000 and May through July 2001 (Table VI.C.12). Adverse weather conditions and plant dormancy precluded sampling in the intervening months. An additional observation was made in August 2002 to further assess the long-term growth differences of the test genotypes.

The mean plant diameter of ASR368 R0 plants and the three commercial creeping bentgrass cultivars was consistently greater than B99061R on all sampling dates (Table VI.C.12). The relatively poor plant vigor of B99061R in comparison to ASR368 and the commercial cultivars suggests that the vegetative plugs planted were not particularly vigorous in this experiment. The mean plant diameter of ASR368 R0 fell within the range of values for the commercial cultivars for every rating over the 25 months or three growing seasons encompassed by the study. ASR368 R0 was not significantly different from the commercial cultivars throughout the duration of the study other than one instance where it was significantly smaller than Penncross during the final measurement in August 2002. Therefore, we would not expect this event to have any greater fitness or competitive advantage in New Jersey or transition zone turf situations than commercially available creeping bentgrass cultivars.

Table VI.C.12. Mean plant diameter (cm) of ASR368 R0, B99061R and three commercial cultivars, from August 2000 to August 2002 in Middlesex County, NJ.

	2000						2001						2002	
Genotype	**August**		**September**		**October**		**May**		**June**		**July**		**August**	
	centimeters													
	Mean	SD[2]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Penn A-4	6.5	2.6	6.6	3.4	7.8	5.7	4.2	3.8	8.5	7.1	10.2	7.4	4.6	10.1
Crenshaw	6.3	1.8	7.3	3.1	12.8	3.9	14.7	7.7	19.0	8.4	18.6	5.8	6.3	19
Penncross	7.6	2.7	8.3	3.4	12.2	3.4	14.2	7.5	18.6	4.5	21.2	4.0	32.8*	15.2
B99061R	4.7	2.6	4.0*	1.8	6.5	3.5	0.0*[1]	0.0	7.0	7.5	5.2*	5.3	1.0	1.7
ASR368 R0	7.8	0.6	8.8	1.3	11.3	2.5	10.5	5.1	12.5	0.5	13.8	3.5	1.8	3.2
LSD	3.3		3.9		8.3		7.4		5.5		7.8		16.3	

* Mean diameter is significantly different than ASR368 according to Fisher's LSD ($\alpha = 0.05$).

[1] B99061R plants were still dormant during May 2001, which resulted in a 0.0 reading for this observation. The plants resumed vegetative growth prior to the next observation, which enabled calculation of the mean plant diameter in both June and July 2001.

[2] SD = Standard deviation

Union County, Ohio (cool season climate)

The mean plant diameter of ASR368 R0 plants was not significantly different from B99061R and the commercial cultivars throughout the 15 months of the study (Table VI.C.13, Fisher's LSD, $\alpha = 0.05$). The density of the Kentucky bluegrass stand as well as competition from broadleaf weeds, such as dandelion and white clover, resulted in more variation among replications of the same cultivar than were observed between cultivars. This variation contributed to the considerably greater mean plant diameter and standard deviation for ASR368 R0 in May 2001. The individual plant diameter for each replication on this date was 5, 8 and 25 cm. The unusually large 25 cm diameter rating for the third replicate is believed to result from inadvertently including the measurement of bentgrass volunteers not related to the ASR368 R0 plants originally transplanted. This measuring error did not occur on either the preceding or subsequent sampling dates.

Table VI.C.13. Mean plant diameter (cm) of ASR368 R0, B99061R and three commercial cultivars during 2000 and 2001 in Union County, OH

	2000								2001											
Genotype	**Jul**		**Aug**		**Sep**		**Nov**		**Mar**		**Apr**		**May**		**Jun**		**Jul**		**Aug**	
	centimeters																			
	X[1]	SD[3]	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Penn A-4	5.5	1.6	8.8	6.2	7.2	3.2	5.9	1.8	4.7	2	2.9	3.1	5.1	8.2	8.3	8.1	6.9	4.5	8	8.4
Crenshaw	5.5	0.8	5.8	1.6	6.4	1.6	5.8	1.3	5.1	1.9	3.9	2.5	4.6	4.1	3.9	5	5.3	2.2	4.1	4.2
Penncross	5.2	1.1	5.2	1.2	5.8	2.6	5.1	1.3	4.3	1.8	3.8	2.2	4.1	4.9	5.8	8	6.7	3.4	6.5	6.8
B99061R	6.0	1.3	4.8	1.6	5.0	2.6	3.3	1.8	3.3	1.9	1.7	1.4	2.2	2.3	3.3	2.9	3.7	3.8	3.6	3.7
ASR368 R0	5.7	0.2	6.3	1.9	5.0	0.5	5.0	1.8	4.3	2.5	3.2	2.8	12.8	10.7	4	3.5	4.7	4.2	5.8	5.9
LSD	NS[2]		NS		NS		3.7		NS		4.4		NS		NS		NS		NS	

[1] X = Mean

[2] NS = not significantly different from ASR368 according to Fisher's LSD ($\alpha = 0.05$).

[3] SD = Standard deviation

Franklin County, Ohio (cool climate)

The creeping bentgrass commercial cultivars in this study were: Backspin, Crenshaw, Penn A-4 and Penncross. Three other bentgrass species were also evaluated: 'Highland' dryland bentgrass (*A. castellana*), 'SR7100' colonial bentgrass (*A. capillaris*) and 'Streaker' redtop bentgrass (*A. gigantea*) to provide a comparison to other commonly planted bentgrasses.

The mean plant diameter of ASR368 F1 progeny was within the range of B99061R and the three creeping bentgrass commercial cultivars on all sampling dates throughout the 23 months encompassed by this study (Table VI.C.14 – 16). Crenshaw was significantly larger than ASR368 F1 progeny during June 2001, but was not significantly different in all other months. Penncross was significantly larger than ASR368 at the end of the study during August and September 2002.

The other bentgrass reference species were more variable in growth in comparison to ASR368 F1 and the other creeping bentgrass cultivars, which may be attributed to species differences. Colonial and dryland bentgrasses were among the slower growing of all of the bentgrasses in the trial.

Table VI.C.14. Mean plant diameter (cm) of ASR368 F1, B99061R and commercial bentgrass cultivars, during April to September 2001 in Franklin County, Ohio.

	2001											
Cultivar	**April**		**May**		**June**		**July**		**August**		**September**	
	centimeters											
	Mean	SD[5]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Backspin	5.5	1.8	6.8	1.4	4.7	0.8	4.9	1.4	7.7	2.4	7.6	1.8
Crenshaw	5.2	1.2	6.6	1.1	5.7*	1.5	6.0	1.8	7.6	1.7	7.7	2.4
Penncross	5.6	1.1	6.2	0.9	4.3	0.4	5.2	1.7	8.6	3.8	7.6	2.1
Penn A-4	4.9	0.9	6.2	1.0	5.0	0.8	4.6	1.5	6.2	3.6	6.2	3.8
Highland[1]	6.1	3.0	7.5*	1.1	4.0	0.5	3.8	0.3	4.1*	0.7	4.4	1.6
SR7100[2]	3.7	0.8	5.0	0.9	3.9	0.5	3.3	0.4	4.4*	1.3	6.2	1.6
Streaker[3]	6.8*	1.0	8.8*	1.7	4.8	0.7	4.6	2.4	6.1	2.9	6.3	3.1
B99061R	5.0	1.2	6.3	0.5	4.8	0.6	5.3	3.2	7.2	1.0	6.8	3.1
ASR368 F1	5.0	1.2	5.9	1.4	4.3	0.9	5.4	2.1	8.1	1.8	6.9	2.6
LSD	1.6		1.3		1.0		NS[4]		2.6		NS	

* Mean diameter is significantly different from ASR368 on date according to Fisher's LSD ($\alpha = 0.05$).
[1] Highland dryland bentgrass (*Agrostis castellana*)
[2] SR7100 colonial bentgrass (*Agrostis capillaris*)
[3] Streaker redtop bentgrass (*Agrostis gigantea*)
[4] NS = not significantly different than ASR368 according to Fisher's LSD ($\alpha = 0.05$).
[5] SD = standard deviation

Table VI.C.15. Mean plant diameter (cm) of ASR368 F1, B99061R and commercial cultivars, during October 2001 through May 2002 in Franklin County, Ohio.

	2001				2002							
Cultivar	**Oct**		**Dec**		**Jan**		**Mar**		**Apr**		**May**	
	centimeters											
	Mean	SD[5]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Backspin	8.1	2.8	7.9	2.7	8.1	2.7	6.0	1.9	6.6	1.7	8.9	2.4
Crenshaw	9.2	2.8	8.4	2.5	7.6	2.4	6.4	1.3	7.6	1.8	10.8	4.1
Penncross	9.5	2.7	8.9	2.6	7.9	2.8	6.9	2.2	7.7	2.4	10.2	3.6
Penn A-4	7.5	3.8	7.7	3.9	7.3	3.5	5.7	3.0	6.8	3.2	7.0	3.4
Highland[1]	5.6	3.0	5.7	3.4	4.8	3.3	4.6	2.5	3.7*	1.5	2.7*	1.4
SR7100[2]	6.3	2.0	6.6	2.1	5.9	2.0	5.0	1.6	4.7	2.3	4.1*	2.4
Streaker[3]	11.4	3.1	10.4	4.3	9.2	4.2	11.6*	3.6	12.9*	4.5	13.9*	6.2
B99061R	7.3	2.3	7.3	2.1	6.8	2.9	5.2	2.5	6.3	0.8	8.0	2.2
ASR368 F1	7.9	3.0	8.2	3.0	6.7	2.2	5.9	2.3	6.9	2.1	8.6	2.5
LSD	3.0		NS[4]		NS		2.5		2.7		3.6	

* Mean diameter is significantly different from ASR368 on date according to Fisher's LSD ($\alpha = 0.05$).
[1] Highland dryland bentgrass (*Agrostis castellana*)
[2] SR7100 colonial bentgrass (*Agrostis capillaris*)
[3] Streaker redtop bentgrass (*Agrostis gigantea*)
[4] NS = not significantly different than ASR368 according to Fisher's LSD ($p = 0.05$).
[5] SD = standard deviation

Table VI.C.16. Mean plant diameter (cm) of ASR368 F1, B99061R and commercial bentgrass cultivars, during June 2002 to September 2002 in Franklin County, Ohio.

	2002							
Cultivar	**June**		**July**		**August**		**September**	
	centimeters							
	Mean	SD[4]	Mean	SD	Mean	SD	Mean	SD
Backspin	7.9	3.7	7.7	3.6	8.2	1.9	9.5	3.5
Crenshaw	8.9	3.2	8.2	3.7	7.4	3.8	10.3	6.1
Penncross	11.1	2.9	10.4	2.3	11.1*	5.9	13.8*	7.6
Penn A-4	7.0	1.8	8.4	4.0	8.6	4.1	6.2	4.4
Highland[1]	1.3*	1.1	1.4*	1.2	1.8*	0.4	2.2*	0.9
SR7100[2]	3.1*	2.7	3.7*	2.3	3.6	1.9	4.8	2.9
Streaker[3]	13.7*	5.7	11.9	5.9	6.2	2.8	14.4*	5.2
B99061R	10.0	2.3	11.8	2.1	9.7	2.0	9.5	2.2
ASR368 F1	9.7	4.0	7.9	4.2	7.0	3.8	7.8	3.3
LSD	3.3		4.1		3.7		4.9	

* Mean diameter is significantly different from ASR368 on date according to Fisher's LSD (p = 0.05).
[1] Highland dryland bentgrass (*Agrostis castellana*)
[2] SR7100 colonial bentgrass (*Agrostis capillaris*)
[3] Streaker redtop bentgrass (*Agrostis gigantea*)
[4] SD = standard deviation

Marion County, Oregon (cool season)

Observations were taken at the Marion County, Oregon site on 27 dates that spanned 32 months beginning August 2000 and ending February 2003. The mean plant diameter of ASR368 R0 generation plants was greater than B99061R on nearly all measurement dates but differences were not always significant. The three commercial creeping bentgrass cultivars were also consistently larger than B99061R. In comparison to the three commercial cultivars, the mean plant diameter of ASR368 R0 was not significantly different from at least one or more of these cultivars on every measurement date during the 32-month study (Tables VI.C.17 - VI.C.19, Fisher's LSD, α = 0.05).

Table VI.C.17. Mean plant diameter (cm) of ASR368 R0, B99061R and three commercial creeping bentgrass cultivars during 2000 in Marion County, Oregon.

	2000							
Genotype	**August**		**September**		**November**		**December**	
	centimeters							
	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD
Penn A-4	6.6	1.6	4.6	1.4	5.1	1.4	5.6	1.7
Crenshaw	6.7	1.5	4.9	1.0	6.8	1.0	6.7	1.1
Penncross	7.3	0.9	5.5	0.7	5.6	1.7	6.2	0.9
B99061R	6.2*	2.9	4.0	0.5	4.3*	1.4	4.0	0.0
ASR368 R0	9.0	1.3	5.3	0.6	6.7	1.3	6.0	0.9
LSD	2.2		1.5		2.2		2.0	

* Mean diameter is significantly different than ASR368 according to Fisher's LSD (α = 0.05).
[1] SD = Standard deviation

Table VI.C.18. Mean plant diameter (cm) of ASR368 R0, B99061R and three commercial creeping bentgrass cultivars during 2001 in Marion County, Oregon.

	2001																							
Genotype	**Jan**		**Feb**		**Mar**		**Apr**		**May**		**Jun**		**Jul**		**Aug**		**Sep**		**Oct**		**Nov**		**Dec**	
	centimeters																							
	X[1]	SD[2]	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Penn A-4	9.6*	2.4	8.8*	2.4	9.7*	1.3	11.6	2.0	9.9	3.4	11.9	3.4	12.2	2.9	14.1	5.7	12.9	5.4	14.2	5.8	14.9	6.6	18.9	7.6
Crenshaw	10.6	1.8	9.5*	2.1	11.6	3.2	15.0	6.3	14.6	2.0	17.6	3.7	17.1	4.0	17.8	5.6	15.6	3.9	18.1	4.4	15.9	3.4	23.0	3.8
Penncross	10.2*	1.8	11.2	1.8	11.6	1.8	16.6	2.6	17.6	5.7	16.1	3.8	15.3	3.0	19.6	5.6	17.7	3.8	19.7	5.1	17.2	4.6	21.9	6.1
B99061R	7.0*	0.9	7.7*	1.2	6.7*	1.6	9.0	3.8	8.5	1.7	10.0*	1.8	10.7	1.6	12.0	3.9	8.7	2.6	11.2	5.0	10.7	2.1	8.2*	4.6
ASR368	13.7	2.8	13.5	2.0	13.5	2.5	14.3	0.8	13.2	1.4	15.7	3.3	14.5	0.9	18.2	5.3	14.7	2.8	13.8	2.1	13.0	3.5	17.5	4.0
LSD	3.4		3.2		3.5		5.6		6.5		5.2		5.0		7.4		6.0		6.9		6.8		8.9	

[1] X = Mean plant diameter
[2] SD = Standard deviation
* Mean diameter is significantly different from ASR368 according to Fisher's LSD (α = 0.05).

Table VI.C.19. Mean plant diameter (cm) of ASR368 R0, B99061R and three commercial creeping bentgrass cultivars during 2002 and 2003 in Marion County, Oregon.

	2002																				2003	
Genotype	**Jan**		**Feb**		**Mar**		**Apr**		**May**		**Jun**		**Jul**		**Aug**		**Sep**		**Oct**		**Feb**	
	X[1]	SD[2]	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
	cm		Cm		Cm		Cm		Cm		Cm		Cm		Cm		Cm		Cm		cm	
Penn A-4	22.8	9.7	17.3	8.7	13.1	8.0	12.9	7.8	14.7	8.7	16.8	9.7	23.0	13.0	24.5	12.2	26.9	14.0	26.1	14.4	24.7*	13.2
Crenshaw	28.3	6.4	19.4	4.2	14.3	5.0	15.7	7.7	13.7	7.0	15.7	6.7	24.2	9.3	28.1	8.8	27.6	10.9	26.8	12.5	25.6*	12.1
Penncross	19.4	6.3	20.9	7.4	18.9	7.4	19.7	7.8	16.6	7.1	19.9	6.2	28.5	9.2	32.2	8.5	31.3	9.2	31.0	11.6	31.3*	9.2
B99061R	7.8	5.3	7.5	4.4	5.8	3.6	6.3	2.0	6.8	2.4	7.0	0.0	10.7	0.8	14.8	6.0	16.7	3.8	14.2	6.0	18.0	10.3
ASR368	18.2	10.3	12.7	2.5	13.5	4.6	13.7	2.5	11.0	1.3	13.2	3.8	19.7	6.8	20.3	4.3	22.5	5.3	21.2	9.0	10.8	2.3
LSD	10.6		8.4		8.2		8.8		8.9		9.2		12.9		13.1		13.9		15.1		13.7	

[1] X = Mean plant diameter
[2] SD = Standard deviation
* Mean diameter is significantly different from ASR368 according to Fisher's LSD ($\alpha = 0.05$).

C.2.a.3. Conclusion of competitive cool season and transition zone relative growth studies

The relative growth and competitive ability of ASR368 as measured by plant diameter was examined in three managed cool season environments and a transition zone turfgrass environment. ASR368 R0 plants were evaluated at three locations and ASR368 F1 plants at one location (Franklin County, Ohio). At three of the four locations the mean plant diameter of ASR368 plants was consistently larger than B99061R. On several dates this difference was statistically significant according to Fisher's LSD ($\alpha = 0.05$). However, the mean plant diameter of ASR368 was not significantly different from at least one of the commercial cultivars on sampling dates spanning 12 to 32 months.

Event ASR368 was selected from among more than 400 transformation events because of its commercially acceptable agronomic, genotypic and phenotypic characteristics. The ASR368 R0 was then crossed with a number of plants derived from commercial cultivars (Elite Parent Plants) to produce the ASR368 F1 progeny population. The same forward breeding process is used to develop the commercial bentgrass cultivars used as controls in these experiments. B99061R was randomly selected amongst other callus tissue derived from other explant genotypes and regenerated for use as an experimental control. It was not possible to go through the same selection and forward breeding process to ensure it was identical and as commercially acceptable as ASR368 or the other commercial bentgrasses except for the expression of the *cp4 epsps* gene. Somaclonal variation among plants regenerated from tissue culture in terms of morphology, growth habit, etc. is well documented in the scientific literature (Evans *et al.* 1984; Fluminhan *et al.* 1996; Muller *et al.* 1990). Consequently, the difference in growth between plants of B99061R and ASR368 may more likely be attributed to somaclonal variation, the event

selection criteria and/or the forward breeding process rather than to the insertion of the *cp4 epsps* gene.

In summary, the mean plant diameter of ASR368 tended to fall within the range of the commercial cultivars at each of the four locations. It is not expected that plants of ASR368 would possess any greater ability to persist than commercially available bentgrass cultivars in competitive cool season or transition zone turfgrass environments. Therefore, it is not expected that ASR368 would pose additional pest risk in managed environments compared to commercial available creeping bentgrass cultivars that are representative of *A. stolonifera*.

C.2.b. Relative growth of ASR368 plants in competitive and managed warm season turfgrass stands.

C.2.b.1. Experimental methods

The plant growth rate of ASR368 F1 was compared to B99061R and Backspin (2001 only), Crenshaw, Penn A-4, Penncross and Seaside (2002 only) creeping bentgrasses in a managed, competitive, warm season turfgrass environment in both 2001 and 2002. Three other bentgrass species were also evaluated: 'Highland' dryland bentgrass, 'SR7100' (2001) and 'Bardot' (2002) colonial bentgrasses and 'Streaker' redtop bentgrass, to provide a comparison to other commonly planted bentgrasses (Table VI.C.1). Vegetative plants of the bentgrass cultivars (nine per cultivar per environment) originating from Marion County, Oregon (as described above in Section VI.C.2.b.1), were transplanted into a mature stand of St. Augustinegrass (*Stenotaphrum secondatum*) in the shade and bermudagrass (*Cynodon dactylon*) in full sun in Baldwin County, Alabama on November 2, 2000 and again November 2, 2001. The plots were watered as needed to prevent moisture stress on the transplanted material. The competitive ability of the creeping bentgrass events and commercial cultivars was recorded monthly by measuring the average diameter of each creeping bentgrass patch.

Statistical analysis

In both years the study was conducted as a completely randomized design in three replicates as described above in Section VI.C.2.a.1. Analysis of variance was used to determine differences in growth among the bentgrass lines. Means were compared using Fisher's LSD ($\alpha = 0.05$).

C.2.b.2. Results

St. Augustinegrass, shade

The mean plant diameter of ASR368 F1 was within the range of B99061R and the commercial bentgrass cultivars on all observation dates in both 2001 and 2002. In 2001, ASR368 F1 was consistently larger than B99061R from June to August 2001 (Table VI.C.20). Penncross was significantly larger than ASR368 F1 during all but the March 2001 measurement date. In 2002, ASR368 F1 was significantly smaller than Penncross

on all sampling dates and significantly larger than B99061R on all but the first observation date (Table VI.C.21). As discussed in the previous section, the difference between ASR368 F1 and B99061R may be attributed to somaclonal variation, the event selection criteria and/or the forward breeding process rather than the insertion of the *cp4 epsps* gene.

Table VI.C.20. Mean plant diameter (cm) of *Agrostis* reference species, B99061R and ASR368 F1 during March 2001 to August 2001 in the shade in Baldwin County, Alabama.

	2001											
Cultivar	**March**		**April**		**May**		**June**		**July**		**August**	
	centimeters											
	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Backspin	22.1	3.3	22.9	3.1	22.6*	3.3	23.7	4.3	23.1	4.4	23*	4.8
Crenshaw	19.4	1.9	21.7	1.7	21.1	1.8	22.1	2	21.1	2.8	20.1	3.1
Highland	21.0	2.4	20.7	3.1	20.0	3.3	21.9	4.2	20.1	3.2	20.7	3.4
Penncross	22.1	2.7	24.6*	3.7	23.7*	2.9	26.8*	3.4	25.8*	2.7	24.3*	4.9
Penn A-4	18.3	2.6	20.8	2.1	19.8	2.4	20.5	2.5	19.6	3.9	19.9	3.6
SR7100	18.9	2.4	20.7	2.5	21.1	2.4	23.1	3.2	22.6	2.8	22.3*	3.7
Streaker	24.8*	4.8	24.8*	5	21.0	3.6	21.4	3	19.8	3.6	19.4	3.1
B99061R	18.3	2.4	18.4	3.9	18.3	1.3	18.2	1.5	17.5	1.4	11.9	10
ASR368 F1	19.1	3.8	20.5	2.2	19.3	1.8	20.7	2.1	19.4	2.1	16.8	6.7
LSD	3.2		3.5		3.2		3.5		3.7		5.0	

* Mean diameter is significantly different from ASR368 according to Fisher's LSD ($\alpha = 0.05$).
[1] SD = Standard deviation

Table VI.C.21. Mean diameter (cm) of creeping bentgrass plants of ASR368 F1, B99061R and commercial cultivars in the shade from April 2002 to August 2002 in Baldwin County, Alabama.

	2002									
Genotype	**April**		**May**		**June**		**July**		**August**	
	centimeters									
	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bardot	20.6*	3.5	19.8	3.9	21.1	3.9	22.0	3.8	19.9	4.1
Crenshaw	19.4	2.5	18.9	2.7	22.1	3.0	22.1	2.2	21.0	3.0
Highland	18.3	4.3	16.0	2.8	18.2	4.4	18.9	5.1	19.6	4.1
Penn A-4	18.5	2.3	18.9	2.6	21.4	3.0	22.5	3.4	20.4	3.5
Penncross	20.6*	2.1	20.4*	2.2	23.5*	3.2	24.7*	3.4	23.1*	3.6
Seaside	19.5	1.9	19.4	3.2	21.5	3.3	22.5	3.8	21.3	4.5
Streaker	20.0*	5.5	18.5	7.1	19.5	7.6	19.1	6.1	17.0	5.6
B99061R	15.2	1.9	13.6*	2.2	15.3*	1.3	15.4*	1.8	15.3*	2.0
ASR368 F1	17.1	1.1	16.9	2.2	19.2	2.5	20.4	3.0	19.5	3.4
LSD (0.05)	2.8		3.2		3.5		3.5		3.5	

* Mean diameter is significantly different from ASR368 according to Fisher's LSD ($\alpha = 0.05$)
[1] SD = Standard deviation

Bermudagrass, full sun

The mean plant diameter of ASR368 F1 plants fell within the range of the mean diameter of B99061R or the commercial bentgrass cultivars and species growing in competition with bermudagrass in full sun in both 2001 and 2002 in Baldwin County, Alabama. In 2001, ASR368 F1 was not significantly different from the commercial creeping bentgrass cultivars on most dates except for two when it was significantly smaller than Crenshaw (March) and Penncross (June) (Table VI.C.22). ASR368 F1 was significantly smaller than Streaker from March through June 2001 but not by the end of the study. ASR368 F1 was also significantly smaller than SR7100 in June and August 2001. In 2002, ASR368 F1 was not significantly different from the commercial creeping bentgrass cultivars on most dates except for two when it was significantly smaller than Crenshaw (April; and June) and Seaside (June) (Table VI.C.23). ASR368 F1 was significantly larger than B99061R on all dates other than for the final observation (Table VI.C.23).

Table VI.C.22. Mean plant diameter (cm) of ASR368 F1, B99061R and commercial bentgrass cultivars during March 2001 to August 2001 in full sun in Baldwin County, Alabama.

	2001											
	March		April		May		June		July		August	
	centimeters											
Genotype	Mean	SD[2]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Backspin	17.1	2.6	15.8	2.6	15.6	2.0	15.1	1.6	13.4	1.2	11.7	1.0
Crenshaw	18.8*	2.3	17.2	2.8	16.5	1.1	15.8	1.7	13.9	1.1	12.9	1.4
Highland	16.5	4.4	16.2	4.0	15.7	3.9	15.5	3.7	12.8	1.4	12.7	2.3
Penncross	17.0	2.2	16.4	1.7	16.8	1.6	16.9*	1.7	14.0	1.8	12.8	1.2
Penn A-4	13.5	2.5	14.5	3.6	14.3	1.8	13.5	2.5	12.6	1.6	12.3	1.2
SR7100	17.0	2.3	16.2	2.2	16.1	2.1	16.3*	1.4	14.8	2.2	13.7*	0.6
Streaker	18.6*	3.4	20.2*	2.2	18.6*	3.2	16.9*	3.0	14.1	1.5	11.6	0.9
B99061R	15.7	1.9	16.0	2.1	14.9	0.8	14.3	1.6	11.7	0.6	11.8	0.3
ASR368 F1	15.2	1.7	13.8	3.3	14.6	1.8	13.6	1.4	13.1	2.5	12.3	1.1
LSD (0.05)	3.1		3.7		2.7		2.4		NS[1]		1.4	

* Mean diameters are significantly different from ASR368 according to Fisher's LSD (α = 0.05).
[1] NS = Mean diameter is not significantly different from ASR368 according to Fisher's LSD (α = 0.05).
[2] SD = Standard deviation

Table VI.C.23. Mean plant diameter (cm) of ASR368 F1, B99061R and commercial bentgrass cultivars in full sun from April 2002 to August 2002 in Baldwin County, Alabama.

	2002									
Genotype	April		May		June		July		August	
	centimeters									
	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bardot	15.6	1.5	14.4	1.6	13.6	0.9	13.3	1.4	12.6	1.5
Crenshaw	16.4*	2.3	13.7	2.6	14.4*	1.8	14.0	1.6	12.1	1.3
Highland	15.3	2.8	13.1	3.1	12.7	1.6	12.8	2.3	10.7	1.3
Penn A-4	12.3	2.7	11.4	1.8	11.8	2.1	11.8	2.4	10.3	1.8
Penncross	13.8	1.4	13.1	2.5	13.6	2.1	12.9	2.0	11.5	1.5
Seaside	15.4	2.5	14.2	2.6	14.5*	2.1	13.5	2.0	11.2	1.1
Streaker'	15.7	3.2	13.7	3.4	13.1	2.7	12.5	1.6	10.3	1.5
B99061R	9.9*	2.8	8.6*	2.5	9.3*	1.6	9.8*	1.8	10.5	1.9
ASR368 F1	14.1	2.0	12.3	1.6	12.3	1.6	12.7	2.4	11.5	1.5
LSD (0.05)	2.2		2.3		1.8		1.8		1.5	

* Mean diameters are significantly different from ASR368 according to Fisher's LSD (α = 0.05).
[1] SD = Standard deviation

C.2.b.3. Conclusion for competitive and managed warm season relative growth studies

No observations were made in either the sun or shade relative growth studies conducted in 2001 and 2002 to indicate that ASR368 F1 plants are any more fit or aggressive than the traditional *Agrostis* cultivars in competition with St. Augustinegrass or bermudagrass in shade or sun, respectively. ASR368 F1 was consistently larger than B99061R in these studies in both 2001 and 2002. However, this difference may be attributed to somaclonal variation, the event selection criteria and/or the forward breeding process rather than the insertion of the *cp4 epsps* gene.

C.2.c. Relative growth under reduced irradiance (shade)

Shade or reduced light quality is a limiting factor in the establishment and persistence of creeping bentgrass in managed and unmanaged ecosystems. Studies were initiated in 2001 and 2002 under low light conditions in Ingham County, Michigan to compare the growth and competitive ability of ASR368 FI with B99061R and several commercial bentgrass cultivars. Commercial cultivars included Backspin, Penn A-4, Crenshaw and Penncross creeping bentgrasses and related species *A. castellana*, 'Highland' dryland bentgrass, *A. capillaris*, 'SR7100' colonial bentgrass and *A. gigantea*, 'Streaker' redtop bentgrass.

C.2.c.1. Experimental methods

<u>Plant propagation and establishment</u>

The creeping bentgrass cultivars or populations were established from stolon nodes or seed and grown in a greenhouse in Oregon prior to shipment to Michigan. Immediately after receipt at the Michigan location, the plants were clipped to 1 inch or less and then transplanted in the indoor shade research facility in Ingham County. The facility is covered with a translucent material that provides 17% to 20% of normal irradiant sunshine in a uniform environment.

Plantings occurred on May 31, 2001 and February 2, 2002 and similar methods were used each time. The plants were set in the soil so that the root and shoot interface (crown) was at or slightly below the soil surface and firm contact between the potting media was maintained with the soil. The trial area was maintained under a nutrient regime appropriate for good plant health and was irrigated every day in the first two weeks of establishment. Thereafter supplemental irrigation was applied three times each week to prevent drought stress for the duration of the trial.

<u>Data collected</u>

The trial was conducted in a randomized complete block design in three replications. The competitive ability of each genotype was evaluated by measuring the ground cover of each creeping bentgrass patch. Ground cover was measured by centering a grid with

121 points on the plant, counting the number of points corresponding with green tissue and then calculating the mean number of points with green tissue.

Statistical analysis

An analysis of variance and least significant difference (LSD) test at the $\alpha = 0.05$ level were used to make multiple comparisons of the mean ground cover of the different genotypes in both 2001 and 2002.

C.2.c.2. Results

ASR368 F1 was intermediate to the creeping bentgrass cultivars, Backspin, Penn A-4, Crenshaw and Penncross and the tissue culture line B99061R, during all measurement dates in 2001 (Table VI.C.24). ASR368 F1 progeny and the commercial creeping bentgrass cultivars were significantly larger than Highland dryland bentgrass, Streaker redtop bentgrass and SR 7100 colonial bentgrass on all dates.

In the 2002 trial, ASR368 F1 progeny were not significantly different from and within the range of B99061R and the four commercial creeping bentgrass cultivars, Backspin, Crenshaw, Penn A-4 and Penncross, during all measurement dates (Table VI.C.25). Also similar to 2001 trial, the Streaker, Highland and SR7100 were consistently among those cultivars with the lowest ground coverage/plant.

C.2.c.3. Conclusions of reduced irradiance relative growth studies

ASR368 F1 progeny population plants were generally intermediate in ground cover compared to those of B99061R or the other commercial creeping bentgrass cultivars in both the 2001 and 2002 evaluations. Therefore, it can be concluded that the vegetative growth of ASR368 progeny in either managed or unmanaged ecosystems under reduced light conditions is not expected to be different from commercial creeping bentgrass.

Table VI.C.24. Comparative growth as measured by mean ground cover of ASR368 F1 progeny, B99061R and *Agrostis* reference genotypes under reduced irradiance conditions in 2001 in Ingham County, Michigan in 2001

	Mean Ground Cover[1]		
Genotype	**2001**		
	June 28	**July 12**	**September 3**
Streaker	18.7d*	17.7e	17.7bc
Highland	24.3d	15.7e	14.7c
SR 7100	31.0d	25.0de	3.3c
B99061R	44.3c	31.0cd	41.0ab
ASR368 F1	47.7c	45.0ab	49.3a
Penn A-4	50.3bc	36.3bcd	45.0a
Backspin	50.7bc	43.3abc	45.0a
Penncross	61.3ab	47.3ab	58.7a
Crenshaw	64.0a	53.3a	53.0a
LSD (α= 0.05)	12.47	13.33	25.22
SD[2]	7.2	7.7	14.57
CV[3]	16.52	22.03	40.02

[1] Mean ground cover is based on total points out of a maximum value of 121 points/ft^2
[2] SD = Standard Deviation
[3] CV = Coefficient of Variation
* Means followed by same letter within dates are not significantly different (α = 0.05, Student-Newman-Keuls)

Table VI.C.25. Comparative growth as measured by ground cover of ASR368 F1 progeny, B99061R and *Agrostis* reference genotypes under reduced irradiance conditions in Ingham County, Michigan in 2002.

	Mean Ground Cover[1]				
Genotype	**2002**				
	February 22	**March 14**	**April 4**	**April 17**	**May 17**
ASR368 F1	1.3a*	4.4ab	15.8ab	19.1a	36.7ab
B99061R	1.6a	4ab	17.7ab	22.2a	29.8bc
Streaker	1.0a	2.6b	7.2b	8b	14.2cd
Penn A-4	1.7a	4.8ab	18.8a	22.8a	48.4ab
Highland	1.0a	2.4b	7.7b	8.2b	8.7d
Backspin	1.6a	4.4ab	17.2ab	21.9a	47ab
SR7100	1.4a	4.1ab	15.3ab	15.2ab	19.2cd
Penncross	1.2a	4.2ab	20.1a	20.1a	47.6ab
Crenshaw	1.2a	5.0a	20.1a	21.8a	54.3a
LSD ($\alpha = 0.05$)	0.44	1.49	7.27	7.2	13.95
SD	0.25	0.86	4.2	4.16	8.06
CV	18.87	21.45	27.03	23.48	23.71

[1] Mean ground cover is based on total points out of a maximum value of 121 points/ft^2
[2] SD = Standard Deviation
[3] CV = Coefficient of Variation
* Means followed by same letter within dates are not significantly different ($\alpha = 0.05$, Student-Newman-Keuls)

C.3. Overall conclusion for relative growth

ASR368 and its progeny displayed no increase in vegetative growth, aggressiveness, invasiveness or relative fitness compared to commercial creeping bentgrass cultivars when established in bare soil with no competition or with competition from other turfgrasses in cool, warm or transition climate zones. ASR368 also demonstrated no competitive advantage in direct sun or shade or reduced light (Ingham County, MI and Baldwin County, AL).

There are a number of reports in the scientific literature confirming that the variability in creeping bentgrass growth observed in these studies was less than that typically observed due to differences in climate and cultural practices, such as mowing, irrigation, and fertilization (Turgeon, 1985; Beard, 1973; Holt and Payne, 1951). Therefore, given the results from these experiments conducted in twelve diverse environments over three years, which further confirm reports in the scientific literature, ASR368 and its progeny would not be expected to grow in a different manner in either managed or unmanaged ecosystems from commercial creeping bentgrass cultivars representative of *A. stolonifera*. Furthermore, these findings support a conclusion of no contribution to increased weed potential of ASR368 based on its growth relative to commercial creeping bentgrass cultivars that are representative of *A. stolonifera*.

D. Flowering

Changes in the reproductive processes of flowering or inflorescence formation may be an unintended effect of the plant transformation process. Several flowering characteristics could enhance the relative fitness of a given species. For example, a creeping bentgrass variety that sets flowers earlier in the season or has a longer flowering period may have enhanced reproductive potential. Characteristics such as heading date, anthesis initiation date, anthesis duration and maturity date of ASR368 relative to other creeping bentgrasses were evaluated in various environments. This section reviews the relative floral development of ASR368 in greenhouse studies conducted at Iowa State University in 2001 and 2002 and field studies conducted in Franklin County, Washington during 2000 and 2001, and Jefferson County, Oregon in 2002 (USDA # 00-220-02n, 01-017-14n, 01-177-01n and 00-177-02n).

D.1. Greenhouse studies

D.1.a. Experimental methods

Genotypes

In 2001, the time to first inflorescence emergence (days to heading, heading date), anthesis initiation and anthesis duration of the ASR368 R0 and F1 progeny were compared to that of B99061R, and plants of the commercial cultivars Penncross, Penn A-4 and Crenshaw. In 2002, ASR368 R0 plants and F1 and F2 progeny populations were compared to plants of B99061R, Penncross, Penn A-4 and Crenshaw.

Plant Propagation

2001 Study: Plants of R0 generation ASR368, B99061R and the commercial cultivars were established in 6" pots from vegetative stolon nodes during September 2000. ASR368 F1 progeny were derived from seed harvested from maternal Elite Parent Plants (EPP) pollinated by ASR368 R0 in a crossing block during summer, 2000. The ASR368 F1 plants were established from seed and maintained similar to the other plant material during September 2000. All R0 and F1 ASR368 plants were hemizygous and dominant for the *cp4 epsps* gene. All plants were propagated and grown to maintain similar plant age across genotypes within each experiment. All plants were maintained in a cold frame poly-house in Marion County, Oregon for development, vernalization and floral induction prior to overnight shipment to Iowa.

Approximately each week beginning in March 2001 and ending in May 2001, one set (batch) of plants was shipped from vernalization and floral induction conditions (natural day length, 0 to 10^0 C) in Oregon to floral initiation conditions (16 hr light, 20 to 25^0 C) in the greenhouse in Iowa. Plants were irrigated as needed to prevent plant moisture stress. Once moved into the greenhouse, irrigation was performed twice per week and greenhouse temperature was set at 20 to 21^0 C.

Plants were fertilized to maintain good plant vigor and health and to avoid nutrient deficiency symptoms. Insecticides and fungicides were applied as needed following standard agronomic practices to control pests.

Plants shipped with each batch from Oregon to Iowa in 2001 are listed below:

- three clones of ASR368 R0;
- three random ASR368 F1 progeny plants;
- three clones of B99061R; and
- five random plants each of Penncross, Crenshaw and Penn A-4.

2002 Study: Plants of ASR368 R0, B99061R and the commercial cultivars were established from vegetative stolon nodes during September 2001. ASR368 F1 plants were derived from seed harvested from maternal EPPs pollinated by ASR368 R0 in a crossing block during summer, 2001. Similarly, ASR368 F2 plants were derived from seed harvested from maternal EPPs pollinated by ASR368 F1 plants in a crossing block during summer, 2001. Both F1 and F2 progeny plants were established from random seeds. All ASR368 R0, F1 and F2 plants are hemizygous dominant for the *cp4 epsps* transgene. All plants were propagated and grown to maintain similar plant age across genotypes within each experiment. All plants were maintained in a cold frame poly-house in Marion County, Oregon for development, vernalization and floral induction prior to overnight shipment to Iowa.

Approximately each week beginning in March 2002 and ending in June 2002, one set (batch) of plants was shipped from vernalization and floral induction conditions (natural day length, 0 to 10^0 C) in Oregon to floral initiation conditions (16 hr light, 20 to 25^0 C) in the greenhouse in Iowa. Plants were maintained as in 2001.

Plants shipped with each batch from Oregon to Iowa in 2002 are listed below:

- three clones of the ASR368 R0 plant;
- five random ASR368 F1 progeny plants;
- five random ASR368 F2 progeny plants;
- three clones of B99061R; and
- five random plants each of three commercial cultivars: Penncross, Crenshaw and Penn A-4.

Data Collected (2001 and 2002)

Inflorescence initiation (first head date) for a plant was determined as the number of days required from the initial placement in floral initiation conditions in the Iowa greenhouse to the emergence of the first three panicles on a plant.

Anthesis date was determined as the number of days required from the initial placement in floral initiation conditions in the greenhouse to the date when the first three panicles on a plant had anthers exterted from the floret.

The duration of anthesis was defined as the number of days from the beginning of anthesis to the end of anthesis for each genotype. End of anthesis was identifed as the day when no additional panicles were observed shedding pollen.

Statistical analysis

The data analysis for both the 2001 and 2002 studies was performed using SAS (version 8.1, the SAS Institute, Cary, NC). The effect of genotype was fixed and the effect of block (different batches) was random in these experiments. Genotype means were calculated taking into consideration block effects and a mixed model was used for analyzing the data. Various comparisons were constructed to determine if the ASR368 genotypes were significantly different from B99061R or any of the commercial cultivars.

D.1.b. Results of greenhouse studies

Number of days required for inflorescence emergence (first head date)

The mean number of days required for inflorescence emergence (first head date) for the five batches (replications) of each genotype or population tested in 2001 and the specific comparisons of the control cultivars with ASR368 R0 and F1 are presented in Tables VI.D.1 and VI.D.2. The mean days to first head date for both ASR368 R0 and F1 were not significantly different from B99061R and Penn A-4. However, significantly more days were required to first head date by Crenshaw and Penncross than by ASR368 F1 progeny. Although Penncross took significantly longer to reach first head date than the ASR368 R0 plants, the days required for Crenshaw was not significantly different from that of ASR368 R0 plants.

Tables VI.D.3 and V.I.D.4 depict the mean number of days required for inflorescence emergence for the five batches of each genotype tested in 2002 and the specific comparisons of the reference genotypes and cultivars with R0, F1 and F2 ASR368. As in 2001, the mean number of days to first head date for all plants derived from ASR368 was not significantly different from B99061R or Penn A-4. In addition, Penncross was not, while Crenshaw was, significantly different from ASR368 R0, F1 and F2 plants for days to first head date.

Table VI.D.1. Number of observations, mean, standard deviation and the minimum and maximum values for the number of days required for first head date among ASR368 R0 and F1 progeny, B99061R and the three commercial cultivars grown in the greenhouse in 2001.

Genotype	Number of observations	Mean (days)	Std deviation	Minimum value	Maximum value
Penn A-4	25	18.11	4.50	9	28
Crenshaw	25	18.92	3.93	13	28
Penncross	25	19.80	2.96	14	26
B99061R	15	16.40	2.67	11	19
ASR368 R0	15	17.33	3.15	13	25
ASR368 F1	15	16.92	4.56	10	29

Table VI.D.2. Comparisons between ASR368 R0 or F1 progeny and B99061R or the three commercial cultivars for first head date in 2001.

Contrast	Mean Difference	StdErr	Probt
B99061R vs. ASR368 R0	16.40 - 17.33 = -0.93	1.09	0.40
B99061R vs. ASR368 F1	16.40 - 16.92 = -0.52	0.99	0.60
Penn A-4-vs. ASR368 R0	18.11 - 17.33 = 0.78	0.92	0.40
Penn A-4 vs. ASR368 F1	18.11 - 16.92 = 1.19	0.80	0.14
Crenshaw vs. ASR368 R0	18.92 - 17.33 = 1.59	0.90	0.08
Crenshaw vs. ASR368 F1	18.92 - 16.92 = 2.00	0.78	0.01*
Penncross vs. ASR368 R0	19.80 - 17.33 = 2.47	0.91	0.01*
Penncross vs ASR368 F1	19.80 - 16.92 = 2.88	0.79	0.0004*

* Means are significantly different ($\alpha = 0.05$)

Table VI.D.3. Number of observations, mean, standard deviation and the minimum and maximum values for the number of days required for first head date among ASR368 R0 and F1 and F2 progeny and B99061R and the three commercial cultivars grown in the greenhouse in 2002.

Genotype	Number of observations	Mean (days)	Std deviation	Minimum value	Maximum value
Penn A-4	5	22.0	1.9	20	24
Crenshaw	5	27.8	4.4	20	30
Penncross	5	24.2	4.1	21	31
B99061R	3	18.7	1.5	17	20
ASR368 R0	3	19.3	4.0	17	24
ASR368 F1	5	22.4	1.8	20	25
ASR368 F2	5	20.8	4.1	14	24

Table VI.D.4. Comparisons between ASR368 R0 and F1 or F2 progeny and B99061R or the three commercial cultivars for first head date in 2002.

Contrast	Mean Difference	StdErr	Probt
B99061R vs. ASR368 R0	18.7 – 19.3 = -0.7	2.76	0.81
B99061R vs. ASR368 F1	18.7 – 22.4 = -3.7	2.47	0.14
B99061R vs. ASR368 F2	18.7 – 24.2 = -2.1	2.47	0.40
Penn A-4-vs. ASR368R0	22.0 – 19.3 = 2.7	2.47	0.29
Penn A-4 vs. ASR368 F1	22.0 – 22.4 = -0.4	2.14	0.85
Penn A-4 vs. ASR368 F2	22.0 – 20.8 = 1.2	2.14	0.58
Crenshaw vs. ASR368 R0	27.8 – 19.3 = 8.5	2.47	0.002*
Crenshaw vs. ASR368 F1	27.8 – 22.4 = 5.4	2.14	0.02*
Crenshaw vs. ASR368 F2	27.8 – 20.8 = 7.0	2.14	0.003*
Penncross vs. ASR368 R0	24.2 – 19.3 = 4.9	2.47	0.06
Penncross vs. ASR368 F1	24.2 – 22.4 = 1.8	2.14	0.41
Penncross vs. ASR368 F2	24.2 – 20.8 = 3.4	2.14	0.13

* Means are significantly different ($\alpha = 0.05$)

Number of days required for anthesis initiation:

Tables VI.D.5 and VI.D.6 contain the mean number of days required for anthesis initiation for the five batches (replications) of each genotype or population tested in 2001 and the specific comparisons between the ASR368 generation treatments and B99061R or the commercial cultivars. Tables VI.D.7 and VI.D.8 contain similar information for the 2002 experiment.

Plants derived from ASR368 fell within the range of the commercial creeping bentgrass cultivars for days required to reach anthesis in both 2001 and 2002. In 2001, the ASR368 R0 and F1 were not significantly different from B99061R, Penncross and Crenshaw but Penn A-4 required significantly fewer days for anthesis initiation than the ASR368 R0. In 2002, plants of ASR 368 R0, F1 and F2 were not significantly different from B99061R, Penncross and Penn A-4, but Crenshaw required significantly more days for anthesis initiation than the ASR368 R0 plants and the F1 and F2 progeny populations.

Table VI.D.5. Number of observations, mean, standard deviation and the minimum and maximum values for the number of days required for anthesis initiation among ASR368 R0 and F1 progeny, B99061R and the three commercial cultivars grown in the greenhouse in 2001.

Genotype	Number of observations	Mean estimate (days)	Std deviation	Minimum value	Maximum value
Penn A-4	25	30.09	4.13	20	39
Crenshaw	25	30.71	3.93	26	43
Penncross	25	31.62	2.98	23	37
B99061R	15	32.07	2.19	25	34
ASR368 R0	15	32.8	2.51	29	37
ASR368 F1	15	30.81	5.25	20	45

Table VI.D.6. Comparisons between ASR368 R0 or F1 progeny and B99061R and the three commercial cultivars for number of days required for anthesis initiation in 2001.

Contrast	Mean Difference	StdErr	Probt
B99061R vs. ASR368 R0	32.07 - 32.80 = -0.73	1.39	0.59
B99061R vs. ASR368 F1	32.07 - 30.81 = 1.26	1.24	0.31
Penn A-4-vs. ASR368 R0	30.09 - 32.80 = -2.71	1.16	0.02*
Penn A-4 vs. ASR368 F1	30.09 - 30.81 = -0.72	0.99	0.47
Crenshaw vs. ASR368 R0	30.71 - 32.80 = -2.09	1.12	0.07
Crenshaw vs. ASR368 F1	30.71 - 30.81 = -0.10	0.96	0.92
Penncross vs. ASR368 R0	31.62 - 32.80 = -1.18	1.14	0.30
Penncross vs. ASR368 F1	31.62 - 30.81 = 0.81	0.98	0.41

* Means are significantly different ($\alpha = 0.05$)

Table VI.D.7. Number of observations, mean, standard deviation and the minimum and maximum values for anthesis initiation among ASR368 R0 and F1 and F2 progeny, B99061R and the three commercial cultivars grown in the greenhouse in 2002.

Genotype	Number of observations	Mean (days)	Std deviation	Minimum value	Maximum value
Penn A-4	5	30.6	1.7	28	32
Crenshaw	5	38.8	5.0	30	42
Penncross	5	33.2	5.1	30	42
B99061R	3	31.0	2.0	29	33
ASR368 R0	3	29.0	3.0	26	32
ASR368 F1	5	30.6	1.5	29	33
ASR368 F2	5	29.0	2.9	25	33

Table VI.D.8. Comparisons between ASR368 R0 and F1 or F2 progeny and B99061R and the three commercial cultivars for number of days required for anthesis initiation in 2002.

Contrast	Mean Difference	StdErr	Probt
B99061R vs. ASR368 R0	31.0 – 29.0 = 2.0	2.80	0.48
B99061R vs. ASR368 F1	31.0 – 30.6 = 0.4	2.50	0.87
B99061R vs. ASR368 F2	31.0 – 29.0 = 2.0	2.50	0.43
Penn A-4-vs. ASR368 R0	30.6 – 29.0 = 1.6	2.50	0.53
Penn A-4 vs. ASR368 F1	30.6 – 30.6 = 0.0	2.17	1.00
Penn A-4 vs. ASR368 F2	30.6 – 29.0 = 1.6	2.17	0.47
Crenshaw vs. ASR368 R0	38.8 – 29.0 = 9.8	2.50	0.0007*
Crenshaw vs. ASR368 F1	38.8 – 30.6 = 8.2	2.17	0.0009*
Crenshaw vs. ASR368 F2	38.8 – 29.0 = 9.8	2.17	0.0001*
Penncross vs. ASR368 R0	33.2 – 29.0 = 4.2	2.50	0.11
Penncross vs. ASR368 F1	33.2 – 30.6 = 2.6	2.17	0.24
Penncross vs. ASR368 F2	33.2 – 29.0 = 4.2	2.17	0.07

* Means are significantly different ($\alpha = 0.05$)

Number of days from the beginning of anthesis to the end of anthesis of each genotype (anthesis duration)

ASR368 and its progeny were not significantly different from B99061R and at least one of the three commercial cultivars for duration of anthesis in both the 2001 and 2002 experiments. Tables VI.D.9 and Table VI.D.10 depict the mean number of days for anthesis duration for the five batches (replications) of each genotype tested and the specific comparisons between the ASR368 treatments with B99061R and the commercial cultivars in 2001. Tables VI.D.11 and VI.D.12 contain the same information for the experiment conducted in 2002. The p-values ($\alpha = 0.05$) indicate that the duration of anthesis for ASR368 R0 were not significantly different from B99061R or any of the three commercial cultivars in 2001. ASR368 F1 progeny were not significantly different from B99061R or Crenshaw, but were significantly different from Penncross and Penn A-4 during 2001. ASR368 R0, F1 and F2 plants were not significantly different from B99061R or any of the commercial cultivars during 2002.

Table VI.D.9. Number of observations, mean, standard deviation and the minimum and maximum values for anthesis duration among ASR368 R0 and F1 progeny, B99061R and the three commercial cultivars grown in the greenhouse in 2001.

Genotype	Number of observations	Mean (days)	Std deviation	Minimum value	Maximum value
Penn A-4	25	11.77	7.05	3	27
Crenshaw	25	14.59	5.33	8	25
Penncross	25	13.19	6.76	4	28
B99061R	15	12.87	5.32	7	24
ASR368 R0	15	11.60	3.46	6	18
ASR368 F1	14	16.35	5.48	8	26

Table VI.D.10. Comparisons between ASR368 R0 or F1 progeny and B99061R and the three commercial cultivars for number of days required for anthesis duration in 2001.

Contrast	Mean difference	StdErr	Probt
B99061R vs. ASR368 R0	12.87 - 11.60 = 1.27	2.11	0.55
B99061R vs. ASR368 F1	12.87 - 16.35 = -3.48	1.92	0.08
Penn A-4-vs. ASR368 R0	11.77 - 11.60 = 0.17	1.78	0.92
Penn A-4 vs. ASR368 F1	11.77 - 16.35 = -4.58	1.55	0.004*
Crenshaw vs. ASR368 R0	14.59 - 11.60 = 2.99	1.73	0.09
Crenshaw vs. ASR368 F1	14.59 - 16.35 = -1.76	1.5	0.24
Penncross vs. ASR368 R0	13.19 - 11.60 = 1.59	1.76	0.37
Penncross vs. ASR368 F1	13.19 - 16.35 = -3.16	1.53	0.04*

* Means are significantly different ($\alpha = 0.05$)

Table VI.D.11. Number of observations, mean, standard deviation and the minimum and maximum values for anthesis duration among ASR368 R0, F1 and F2 progeny and B99061R and the three commercial cultivars grown in the greenhouse in 2002.

Genotype	Number of observations	Mean (days)	Std deviation	Minimum value	Maximum value
Penn A-4	5	27.4	3.4	23	32
Crenshaw	5	27.6	2.1	25	30
Penncross	5	25.4	7.9	12	32
B99061R	3	25.7	1.2	25	27
ASR368 R0	3	23.3	3.5	20	27
ASR368 F1	5	25.6	1.1	24	27
ASR368 F2	5	25.6	1.8	24	28

Table VI.D.12. Comparisons between ASR368 R0 and F1 or F2 progeny and B99061R and the three commercial cultivars for number of days required for anthesis duration in 2002.

Contrast	Mean Difference	StdErr	Probt
B99061R vs. ASR368 R0	25.7 – 23.3 = 2.3	3.14	0.46
B99061R vs. ASR368 F1	25.7 – 25.6 = 0.07	2.81	0.98
B99061R vs. ASR368 F2	25.7 – 25.6 = 0.07	2.81	0.98
Penn A-4-vs. ASR368 R0	27.4 – 23.3 = 4.1	2.81	0.16
Penn A-4 vs. ASR368 F1	27.4 – 25.6 = 2.0	2.43	0.47
Penn A-4 vs. ASR368 F2	27.4 – 25.6 = 1.84	2.43	0.47
Crenshaw vs. ASR368 R0	27.6 – 23.3 = 4.3	2.81	0.14
Crenshaw vs. ASR368 F1	27.6 – 25.6 = 2.0	2.43	0.42
Crenshaw vs. ASR368 F2	27.6 – 25.6 = 2.0	2.43	0.42
Penncross vs. ASR368 R0	25.4- - 23.3 = 2.1	2.81	0.47
Penncross vs. ASR368 F1	25.4 – 25.6 = -0.2	2.43	0.94
Penncross vs. ASR368 F2	25.4 – 25.6 = -0.2	2.43	0.94

D.2. Field studies

D.2.a. Experimental methods

In 2001, ASR368 R1 seedlings resulting from a cross of ASR368 R0 with Elite Parent Plants known to be segregating for RR and RS phenotypes were evaluated in trials at two locations in Franklin County, Washington (USDA # 00-220-02n and 01-177-02n). The ASR368 R1 population harvested from the R0 plant was expected to segregate in a 1:1 ratio for the RR and RS phenotypes (Figure V.14.).

In 2002, plants of ASR368 F1 and F2 progeny and three commercial creeping bentgrass cultivars Backspin, Crenshaw and Penn A-4 were evaluated at the two locations in Jefferson County, Oregon (USDA #01-177-02N). The ASR368 F1 progeny and the three commercial cultivars were compared in the first study location and the ASR368 F2 progeny and commercial cultivars were evaluated at the second study location.

2000 – 2001 trials in Franklin County, Washington

Plant Propagation

Seedlings were allowed to mature in a greenhouse in Marion County, Oregon until field release. Plantings were made at two locations in Franklin County, Washington. Site I was planted on October 17, 2000 and Site II was planted in on April 30, 2001.

Planting and establishment - Fall Planting

Sixty random ASR368 R1 plants were transferred to the field in Franklin County Washington on October 17, 2000 upon reaching the 2 to 3-tiller stage. The planting consisted of six rows with ten plants each on five foot spacing. Floral induction and vernalization occurred under natural environmental conditions for subsequent floral initiation and seed production during summer 2001.

Planting and establishment - Spring Planting

Plants intended for planting during the spring were transplanted to six-inch plastic pots containing an organic peat potting mix when plants reached the five to six tiller stage during fall 2000. All plants were maintained in Marion County, Oregon for floral induction and subsequently moved to the second field site in Franklin County, Washington on April 30**,** 2001 for floral initiation and seed production during the summer. Planting procedures were similar to the fall 2000 planting.

Determination of RR and RS subpopulations

ASR368 R1 plants in the fall planting (field planted in October 2000) were individually sampled and tested during March to May 2001 for the presence of the *cp4 epsps* gene

using PCR (polymerase chain reaction) techniques. Those plants testing positive for the gene were assigned to the RR sub-population. Those plants testing negative for the presence of the gene were assigned to the RS sub-population. Plants of ASR368 R1 in the spring planting (field planted on April 30, 2001) were individually tested for presence or absence of the CP4 EPSPS protein with a non-destructive immuno-assay strip test (Strategic Diagnostics Inc, Newark, DE).

Data collection

Heading date was recorded at the two Washington locations as the date when the first three seedheads on a plant were exerted from the flag leaf collar. Anthesis date was recorded as the date when the first three seedheads on a plant had florets with anthers exerted from the glumes.

Statistical analysis

The flowering characteristics of the ASR368 R1 RR and RS were analyzed by pooling the data for each characteristic after having accounted for the potential variability contributed by location using Friedman's test ($\alpha = 0.05$).

2001 - 2002 trials in Jefferson County, Oregon

ASR368 F1 trial

ASR368 F1 seed harvested the summer of 2000 was planted in the Marion County, Oregon poly-house in August 2000. The germinated seedlings were sprayed with Roundup Pro herbicide to remove the Roundup susceptible (RS) segregates. Randomly selected ASR368 F1 RR seedlings were then transplanted and maintained in the poly-house until planting in the field the following year. Three commercial creeping bentgrass cultivars were concurrently cultivated from random seedlings and maintained in the same fashion as the ASR368 F1 plants. The ASR368 F1 field trial was field-planted with one-year old plants of all genotype populations.

ASR368 F2 trial

Seed of the ASR368 F2 RR were harvested, planted and selected during the summer of 2001 in the same manner as the F1 progeny in the previous year. The commercial cultivars were also initiated from random seedlings at the same time and maintained the same as the ASR368 F2 plants. The ASR368 F2 trial was field-planted with mature plants with 3 to 4 tillers from each genotype population.

Data collection

Heading date was recorded at the two Oregon locations as the date when the first three seedheads on a plant were exerted from the flag leaf collar. Anthesis date was recorded as the date when the first three seedheads on a plant had florets with anthers exerted from

the glumes. Anthesis end date was documented as the date when the last three seedheads on a plant had florets with anthers exerted from the glumes. Anthesis duration was calculated as the number of days from the beginning of anthesis to the end of anthesis. Panicle maturity date was documented as the date when the majority of the panicles were mature for the harvest process. Maturity duration period was calculated as the number of days from the beginning of anthesis to the panicle (seed head) maturity date.

Statistical analysis

The data from the ASR368 F1 and F2 evaluations were analyzed separately using the Kruskal-Wallis test ($\alpha = 0.05$) to compare the ASR368 flowering characteristics with each commercial cultivar (paired comparison). Fisher's LSD ($\alpha = 0.05$) was used post-hoc for pair-wise testing between groups.

D.2.b. Results of field studies

2000 - 2001

There were no significant differences in the median heading dates between the ASR368 R1 RR and RS segregants in either the fall 2000 or spring 2001 planting locations. There was also no significant effect of planting date/location (site) for heading date of plants. The latest heading dates for the ASR368 R1 RR (June 3) and RS (June 5) segregants differed by just two days. Median heading dates were May 24 for RS R1 progeny and May 25 for RR R1 progeny. The difference was not statistically significant (Table VI.D.13) and unlikely to be of biological significance given the variability found among commercial cultivars during the 2001 - 2002 trials.

The range (earliest to latest) for anthesis initiation date among plants extended three days longer for the ASR368 R1 RR progeny (28 days) versus the RS progeny (25 days). However, median anthesis date for RS progeny (June 13) was 2 days later than for RR progeny (June 11), but the difference was not statistically significant (Table VI.D.14).

Table VI.D.13. Earliest, latest and median heading date for ASR368 R1 RR and RS segregants evaluated during 2001 in Franklin County, Washington

Genotype	n	Earliest date	Latest date	Median date	Day range	P-value	
All RS	46	20-May	03-Jun	24-May	12 days	**Site**	**Segregants**
All RR	67	20-May	05-Jun	25-May	14 days	0.392	0.440

Table VI.D.14. Earliest, latest and median anthesis date for ASR368 R1 RR and RS segregants evaluated during 2001 in Franklin County, Washington

Genotype	n	Earliest Date	Latest Date	Median Date	Day Range	P-value	
All RS	47	01-Jun	25-Jun	13-Jun	25 days	**Site**	**Segregants**
All RR	67	01-Jun	28-Jun	11-Jun	28 days	0.5054	0.5723

2001 - 2002

Heading date for the ASR368 F1 RR population extended from June 2, 2002 to June 12, 2002. The F1 population (mean ~ June 8) was not significantly different from the mean heading dates of Backspin and Crenshaw but was significantly different from Penn A-4 (mean 7.3, Table VI.D.15). The mean heading date for the ASR368 F2 progeny was ~ June 8 and ranged from June 3 to June 12, 2002 was not significantly different from Backspin and Penn A 4, but was significantly different from Crenshaw (Table VI.D.15).

The date anthesis began for ASR368 F1 progeny ranged from June 10 to June 17 with a mean date of June 12, 2002, which was not significantly different from the mean anthesis begin dates of the three commercial cultivar populations (Table VI.D.16). The date anthesis began for ASR368 F2 progeny ranged from June 9 to June 19 with a mean date of June 11, 2002. This duration was not significantly different from Backspin but significantly different from Crenshaw and Penn A 4 (Table VI.D.16).

The date upon which anthesis ended for the ASR368 F1 ranged from July 3 to July 8 with a mean anthesis duration of 24 days, which was not significantly different from the mean days duration for the commercial cultivars (Table VI.D.17). The date anthesis ended for the ASR368 F2 progeny ranged from July 4 to July 9, with a mean anthesis duration of 25.6 days, which was also not significantly different from the commercial cultivars (Table VI.D.17).

The duration of anthesis to seedhead maturity for ASR368 F1 progeny extended from June 12 to July 18, 2002 with a mean duration of 34.4 days. This period was not significantly different from that of Crenshaw and Penn A-4 but significantly different from that of Backspin (Table VI.D.18). The mean duration for anthesis to seedhead maturity for ASR368 F2 extended from July 11 to July 18, 2002 with a mean of 34.6 days. This period was not significantly different from any of the commercial cultivars (Table VI.D.18).

Table VI.D.15. Mean heading date for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars within the month of June 2002 in Jefferson County, Oregon.

Genotype Population	ASR368 F1 Evaluation				ASR368 F2 Evaluation			
	June Heading date				Heading date			
	n	Earliest	Latest	Mean	n	Earliest	Latest	Mean
ASR368	37	Jun-02	Jun-12	Jun-8.5	40	Jun-03	Jun-12	Jun-7.8
Backspin	28	Jun-06	Jun-12	Jun-8.1	38	Jun-06	Jun-12	Jun-7.5
Crenshaw	35	Jun-04	Jun-12	Jun-8.1	38	Jun-02	Jun-10	Jun-6.6*
Penn A-4	25	Jun-06	Jun-08	Jun-7.3*	38	Jun-06	Jun-08	Jun-7.8
p value ($\alpha = 0.05$)				0.032				0.004
LSD ($\alpha = 0.05$)				1.0				0.74

* Means are significantly different from ASR368 F1 or F2 according to Fisher's exact test ($\alpha = 0.05$)

Table VI.D.16. Mean anthesis begin date for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars within the month of June 2002 in Jefferson County, Oregon.

Genotype	ASR368 F1 Evaluation				ASR368 F2 Evaluation			
	Anthesis begin date				Anthesis begin date			
	n	Earliest	Latest	Mean	n	Earliest	Latest	Mean
ASR368	37	Jun-10	Jun-17	Jun-12.2	40	Jun-09	Jun-19	Jun-11.4
Backspin	29	Jun-09	Jun-18	Jun-13.1	38	Jun-09	Jun-18	Jun-11.3
Crenshaw	35	Jun-09	Jun-17	Jun-11.7	37	Jun-09	Jun-15	Jun-10.4*
Penn A-4	25	Jun-09	Jun-18	Jun-11.7	39	Jun-09	Jun-17	Jun-10.5*
p value ($\alpha = 0.05$)				0.14				0.03
LSD ($\alpha = 0.05$)				1.25				0.89

* Means are significantly different from ASR368 F1 or F2 according to Fisher's exact test ($\alpha = 0.05$)

Table VI.D.17. Anthesis ending date and mean anthesis duration in days for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars within the month of July 2002 in Jefferson County, Oregon.

Genotype	ASR368 F1 Evaluation						ASR368 F2 Evaluation					
	Anthesis ending date			Anthesis duration (days)*			Anthesis ending date			Anthesis duration (days)*		
	n	Earliest	Latest	Mean	Min	Max	n	Earliest	Latest	Mean	Min	Max
ASR368	37	Jul-03	Jul-08	24.1	18	27	40	Jul-04	Jul-09	25.6	17	30
Backspin	29	Jul-04	Jul-08	24.1	18	29	38	Jul-04	Jul-09	25.6	19	29
Crenshaw	35	Jul-03	Jul-08	24.8	19	29	38	Jul-03	Jul-09	26.4	21	30
Penn A-4	24	Jul-02	Jul-09	25.5	20	31	39	Jul-03	Jul-09	26.3	21	31
p value ($\alpha = 0.05$)				0.23						0.43		
LSD ($\alpha = 0.05$)				1.40						1.07		

* Anthesis duration = the number of days from the beginning of anthesis to the end of anthesis.

Table VI.D.18. Seedhead maturity (date) and anthesis to seedhead maturity duration[1] (days) for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars within the months of June and July 2002 in Jefferson County, Oregon.

Genotype	ASR368 F1 Evaluation						ASR368 F2 Evaluation					
	Seedhead Maturity Date			Anthesis to Seedhead Maturity (Days)			Seedhead Maturity Date			Anthesis to Seedhead Maturity (Days)		
	n	Earliest	Latest	Mean	Min	Max	n	Earliest	Latest	Mean	Min	Max
ASR368	37	Jul-13	Jul-18	34.4	29	39	39	Jul -11	Jul-18	34.6	26	38
Backspin	29	Jul -11	Jul-17	32.9*	26	38	38	Jul -12	Jul-18	34.3	27	39
Crenshaw	35	Jul -12	Jul-18	35.0	29	39	39	Jul -12	Jul-18	35.7	31	40
Penn A-4	25	Jul -11	Jul-18	33.8	25	39	39	Jul -12	Jul-18	35.5	28	39
p value ($\alpha = 0.05$)				0.05						0.08		
LSD ($\alpha = 0.05$)				1.44						1.15		

[1] The maturity duration period was determined as the number of days from the beginning of anthesis to the seed head maturity date.

* Means are significantly different from ASR368 F1 or F2 according to Fisher's exact test ($\alpha = 0.05$)

D.3. Overall conclusion for flowering

Flowering characteristics of plants derived from ASR368 were compared to several commercial cultivars, B99061R and/or null segregants (RS) in greenhouse and field experiments conducted in 2001 and 2002. No consistently significant differences were detected between ASR368 R0 plants or F1 and F2 RR progeny and non-transgenic conventional creeping bentgrass plants and populations for flowering characteristics including heading date, anthesis initiation dates, anthesis duration or maturity dates. In 2001, ASR368 R0 and F1 RR were not significantly different from B99061R and at least one of the commercial bentgrass cultivars for the days required for heading, anthesis initiation and anthesis duration. In addition, no significant differences between RR and RS progeny derived from ASR368 were observed for heading date or anthesis date in field trials conducted at two locations in Franklin County, Washington during 2001.

In 2002, ASR368 F1 and F2 progeny were not significantly different from B99061R and at least one of the commercial cultivars for beginning head date, anthesis initiation, and duration of anthesis at the two Jefferson County, Oregon locations. ASR368 F1 had a significantly earlier mean heading date than Penn A-4 and required significantly more days from anthesis to seedhead maturity than Backspin. ASR368 F2 had significantly earlier mean heading and anthesis begin dates than Crenshaw.

The scientific literature also provides evidence of considerable variability in the initiation and duration of inflorescence and flowering by species of *Agrostis*. Christoffer (2003) evaluated the flowering characteristics of ASR368 and a number of other *Agrostis* and *Polypogon* species. In this study, which spanned two years, the initiation of anthesis for ASR368 was not different from that of several non-transgenic *A. stolonifera* genotypes in both years evaluated. Christoffer (2003) also cites a number of authors that reported bentgrass anthesis to begin during May and June and end from June to August depending upon environment and species. More specifically, Davies (1953) reported that it takes *A. stolonifera* approximately 22 days from the initiation of first inflorescence to initiation of anthesis. These findings support the results of the greenhouse and field studies presented in this chapter.

The results of the flowering studies presented in this chapter, which are consistent with the scientific literature, demonstrate that the flowering characteristics of ASR368 and its progeny are within the normal range of the commercial creeping bentgrass cultivars or other non-transgenic creeping bentgrasses and should not be expected to flower differently from them. These results also support a conclusion of no contribution to increased weed potential based on the flowering characteristics of ASR368 compared to commercial creeping bentgrass cultivars that are representative of *A. stolonifera.*

E. Pollen Size, Viability and Longevity

Creeping bentgrass is a highly self-incompatible; essentially obligate outcrossing and wind-pollinated species. Within the United States the potential for outcrossing among *Agrostis spp.* is understood and low levels of gene flow have been viewed as an acceptable risk in agricultural seed production (Knowles, 1966). Studies by Wipff and Fricker (2001), Belanger *et al.* (2003) and Christoffer (2003) demonstrate that a low level (ca. 0.02%) of creeping bentgrass outcrossing to conventional creeping bentgrass (*Agrostis stolonifera* to *A. stolonifera*) may occur at distances up to 354 meters from the pollen source. However, the potential for intraspecific or interspecific gene flow is influenced not only by genetic compatibility but also by characteristics of the pollen itself such as its size and duration of viability, i.e. longevity. If these characteristics of ASR368 pollen are altered, these plants may have a greater or lesser ability to outcross with *A. stolonifera* or other species with which it is known to interbreed. Therefore, in this section, the biology of ASR368 pollen is evaluated. To conduct these studies, pollen was collected from the same greenhouse-grown plants used to assess the flowering characteristics in 2001 discussed in the previous section (VI.D.1). Pollen characteristics in 2002 were analyzed from plants vernalized and induced to flower in the field and moved to the greenhouse for final floral initiation and pollen production. These experiments were conducted under USDA # 00-220-02n, 01-017-14n, 01-177-01n and 00-177-02n.

E.1. Experimental methods

<u>Plant propagation and establishment</u>

A detailed description of the genotypes evaluated in 2001 is provided in section VI.D.1.a.

During 2002, the pollen size, viability and longevity of nine plants representing four ASR368 F2 lines and three plants each of the commercial cultivars Backspin, Crenshaw and Penn A-4 were evaluated. These plants were propagated from seed during late summer 2001 and random plants were transplanted to Jiffy pots in September. On September 25, 2001, plants were field-planted in Jefferson County, Oregon. Plants were treated in similar fashion and exposed to natural vernalization conditions and then removed and shipped to Iowa greenhouses on May 30, 2002 when plants were beginning floral initiation. Upon receipt in Iowa, plants were placed in an environment for continued floral initiation similar to that used for greenhouse plants from 2001 studies described in section VI.D.1.a of this petition.

<u>Pollen collection</u>

In both 2001 and 2002, an artificial germination medium containing 0.5 M sucrose, 1 mM boric acid, 2 mM $CaCl_2$ and 0.3% phytogel was used for comparisons of ASR368 creeping bentgrass plants to B99061R and/or commercial cultivars.

Pollen was collected at 11:00 am of the collecting day from inflorescences of three plants of each genotype and placed in an empty Petri plate. One subsample was immediately dusted onto the germination media to determine the initial base-line germination. The remnant pollen was immediately stored and then sub-sampled over time as described below in order to establish the decline of pollen viability over time.

Pollen size

Thirty pollen grains from each of three inflorescences from each plant were measured in 2001 and 2002 using a compound microscope. The diameter of a pollen grain was recorded to the nearest 1 micron.

Pollen storage and germination

In both 2001 and 2002, pollen samples were stored in a desiccator sealed with silicon gel in the dark in a Percival® growth chamber at 21 C. The humidity within the desiccator was adjusted to 67% using saturated $NaNO_2$. Pollen storage conditions were designed to mimic environmental conditions common in the Pacific Northwest during typical dates of creeping bentgrass anthesis. Pollen sub-samples were removed for a germination test every 30 minutes for three hours or until pollen lost viability. Pollen germination was performed at 25^0 C. After 1 hour of germination, Petri plates containing germinating pollen were moved to a refrigerator at 4^0 C to slow further pollen tube elongation in order to simplify counts of germinated pollen later. In most cases during 2001, more than 300 pollen grains were counted for each observation using a Nikon® compound microscope. In 2002, fewer pollen grains were available for this experiment so the pollen from the four F2 progeny lines and the three conventional cultivars was pooled within genotypes.

Statistical analysis

As described in Section VI.D.1.a of this petition.

E.2. Results

Pollen size

The mean, standard deviation, and the minimum and maximum pollen diameter for the ASR368 genotypes, B99061R and the conventional genotypes evaluated in 2001 and 2002 are presented in Table VI.E.1 and VI.E.3, respectively. The specific comparisons in pollen size between plants derived from event ASR368 and the control genotypes in 2001 and 2002 are provided in Tables VI.E. 2 and VI.E.4, respectively. The p-values (α = 0.05) from these comparisons indicate that the size of pollen from ASR368 R0 and F1 plants was not significantly different from that of B99061R or the three commercial creeping bentgrass cultivars in 2001. The pollen diameter of ASR368 F2 plants was not significantly different from that of the three conventional cultivars during 2002.

Table VI.E.1. Number of observations, mean, standard deviation, and minimum and maximum values for pollen diameter (µm) among ASR368 R0 and F1 progeny, B99061R and three commercial cultivars in 2001.

Genotype	Number of observations	Mean diameter	Std deviation	Minimum value	Maximum value
Penn A-4	15	42.6	2.43	38.2	47.8
Crenshaw	15	41.96	1.87	38.3	45.3
Penncross	14	42.09	2.6	36.7	45.2
B99061R	9	42.11	4.09	37.2	47.7
ASR368 R0	9	41.53	3.91	33	46.5
ASR368 F1	9	41.68	3.17	36.3	45.3

Table VI.E.2. Mean difference, standard error and p value (α = 0.05) associated with each comparison between ASR368 R0 or F1 progeny and B99061R and three commercial cultivars for pollen diameter (µm) in 2001.

Contrast	Mean difference	StdErr	Probt
B99061R vs. ASR368 R0	42.11 - 41.53 = 0.58	1.27	0.66
B99061R vs. ASR368 F1	42.11 - 41.68 = 0.43	1.3	0.74
Penn A-4 vs. ASR368 R0	42.60 - 41.53 = 1.07	1.14	0.36
Penn A-4 vs. ASR368 F1	42.60 - 41.68 = 0.92	1.18	0.44
Crenshaw vs. ASR368 R0	41.96 - 41.53 = 0.43	1.14	0.71
Crenshaw vs. ASR368 F1	41.96 - 41.68 = 0.28	1.18	0.81
Penncross vs. ASR368 R0	42.09 - 41.53 = 0.56	1.16	0.63
Penncross vs. ASR368 F1	42.09 - 41.68 = 0.41	1.19	0.73

Table VI.E.3. Mean pollen diameter (µm) of four ASR368 F2 progeny lines[1] and three commercial cultivars evaluated in 2002.

Genotype	Number of observations	Mean diameter (µm)	Std deviation	Minimum value	Maximum value
ASR368 F2 (13-2-2)	3	41.00	4.77	35.5	44.0
ASR368 F2 (14-2-6)	3	37.33	4.16	34.0	42.00
ASR368 F2 (15-2-5)	3	39.67	2.36	37.00	41.5
ASR368 F2 (16-2-2)	3	39.83	2.75	37.0	42.5
Penn A-4	3	40.00	1.80	38.0	41.5
Crenshaw	3	37.17	4.01	33.0	41.0
Backspin	3	38.83	1.76	37.0	40.5

[1] 13-2-2, 14-2-6, 15-2-5 and 16-2-2 refer to the maternal EPP of the respective ASR368 F2 progeny.

Table VI.E.4. Mean difference, standard error and the p value (α = 0.05) associated with each comparison between four ASR368 F2 progeny lines[1] and B99061R and three commercial cultivars for pollen diameter (µm) in 2002.

Contrast	Mean difference	StdErr	Probt
Penn A-4-vs. ASR368 F2 (13-2-2)	40.00-41.00 = -1.0	2.68	0.72
Penn A-4 vs. ASR368 F2 (14-2-6)	40.00-37.33 = 2.7	2.68	0.34
Penn A-4 vs. ASR368 F2 (15-2-5)	40.00-39.67 = 0.3	2.68	0.90
Penn A-4 vs. ASR368 F2 (16-2-2)	40.00-39.83 = 0.2	2.68	0.95
Crenshaw vs. ASR368 F2 (13-2-2)	37.17-41.00 = -3.8	2.68	0.18
Crenshaw vs. ASR368 F2 (14-2-6)	37.17-37.33 = -0.2	2.68	0.95
Crenshaw vs. ASR368 F2 (15-2-5)	37.17-39.67 = -2.5	2.68	0.37
Crenshaw vs. ASR368 F2 (16-2-2)	37.17-39.83 = -2.7	2.68	0.34
Backspin vs. ASR368 F2 (13-2-2)	38.83-41.00 = -2.2	2.68	0.43
Backspin vs. ASR368 F2 (14-2-6)	38.83-37.33 = 1.5	2.68	0.59
Backspin vs. ASR368 F2 (15-2-5)	38.83-39.67 = -0.8	2.68	0.76
Backspin vs. ASR368 F2 (16-2-2)	38.83-39.83 = -1.0	2.68	0.72

[1] 13-2-2, 14-2-6, 15-2-5 and 16-2-2 refer to the maternal EPP of the respective ASR368 F2 progeny.

Pollen viability and longevity

In both 2001 and 2002, the longevity of pollen from plants derived from ASR368 was not significantly different from either B99061R or the commercial cultivars. Minimum and maximum pollen longevity among all populations tested was between 0.5 and 3.5 hours under the conditions provided in the laboratory. Mean longevity was approximately 1.5-2.5 hours. The means, standard deviations and the minimum and maximum values for pollen longevity for the ASR368 genotypes and the three conventional cultivars for the 2001 experiment are presented in Table VI.E.5. The specific comparisons in pollen viability between the ASR368 R0 and F1 and B99061R and the commercial cultivars are provided in Table VI.E.6. The means, standard deviation and the minimum and maximum values for pollen longevity for the ASR368 F2 progeny and the three conventional cultivars for the 2002 experiment are presented in Table VI.E.7.

Table VI.E.5. Number of observations, mean, standard deviation, and the minimum and maximum values for pollen longevity (hours) among ASR368 R0 and F1 progeny, B99061R and three commercial cultivars in 2001.

Genotype	Number of observations	Mean (hours)	Std deviation	Minimum value	Maximum value
Penn A-4	7	1.55	0.85	0.5	3.0
Crenshaw	8	1.95	0.90	0.5	3.0
Penncross	6	1.48	0.98	0.5	3.0
B99061R	6	2.30	1.02	1.0	3.5
ASR368 R0	6	1.84	0.97	0.5	3.0
ASR368 F1	9	1.78	0.71	1.0	3.0

Table VI.E.6. Mean difference, standard error and the p value ($\alpha = 0.05$) associated with each comparison between ASR368 R0 or F1 progeny and B99061R and three commercial cultivars for pollen longevity in 2001.

Contrast	Mean difference	StdErr	Probt
B99061R vs. ASR368 R0	2.30 - 1.84 = 0.46	0.57	0.43
B99061R vs. ASR368 F1	2.30 - 1.78 = 0.52	0.58	0.38
Penn A-4 vs. ASR368 R0	1.55 - 1.84 = -029	0.56	0.61
Penn A-4 vs. ASR368 F1	1.55 - 1.78 = -0.23	0.57	0.68
Crenshaw vs. ASR368 R0	1.95 - 1.84 = 0.11	0.57	0.85
Crenshaw vs. ASR368 F1	1.95 - 1.78 = 0.17	0.58	0.77
Penncross vs. ASR368 R0	1.48 - 1.84 = -0.36	0.58	0.54
Penncross vs. ASR368 F1	1.48 - 1.78 = -0.3	0.59	0.61

Table VI.E.7. Number of observations, mean, standard deviation, and the minimum and maximum values for pollen longevity (hours) among pooled ASR368 F2 progeny lines and three commercial cultivars in 2002.

Group	Number of observations	Mean (hours)	Std deviation	Minimum value	Maximum value
Commercial cultivars	7	2.29	0.64	1.50	3.00
ASR368 F2	6	2.17	0.26	2.00	2.50
Pr > F ($\alpha = 0.05$)		0.68 NS			

NS = no significant differences between groups detected by the F test ($\alpha = 0.05$).

E.3. Conclusions for pollen studies

Considering the consistency of the 2001 and 2002 results of the pollen size, viability and longevity studies, which encompassed three generations of ASR368, it is not expected that pollen of ASR368 varieties would differ in their ability to disperse or outcross with other *A. stolonifera* or other species with which it can interbreed compared to commercial cultivars. The pollen longevity results are in general agreement with reports in the scientific literature regarding other grass species. The longevity of grass pollen is generally considered low. Maize pollen was reported to lose viability after 2 hours under field conditions (Luna et al., 2001) and the pollen viability of Sudan grass (*Sorghum vulgare* var *sudanense*) became negligible 5 hours after pollen shed (Hogg and Ahlgren, 1943).

Consequently, given the results from the ASR368 pollen experiments, which are consistent with reports in the scientific literature regarding the longevity of grass pollen, ASR368 pollen would not be expected to be viable for a longer period of time than pollen from non-transgenic creeping bentgrasses. The results of these studies also indicate that the size of ASR368 pollen would not be expected to be different from pollen of non-transgenic creeping bentgrass. These findings support a conclusion of no contribution to increased weed potential of ASR368 compared to commercial creeping bentgrass cultivars that are representative of *A. stolonifera* based on the size and longevity of its pollen.

F. Fecundity

As discussed in Section II.E.4 of this petition, fecundity refers to both reproductive and/or vegetative success of an organism in nature (Barbour, 1987). Both aspects of fecundity are important when considering *A. stolonifera* because of its predominantly clonal expansion activity in stable environments and seed production under environmental stress. A decrease or increase in seed production may influence the plant's desirability by a seed producer or potentially impact the number of seed available to germinate in managed or unmanaged environments. An increase in seed production might enhance the plants ability to persist and consequently its weed or plant pest potential.

The results from several greenhouse and field experiments conducted in 2001 and 2002 comparing the seed set, seed yield and vegetative biomass of ASR368 and several other commercial creeping bentgrass cultivars are presented in this section. These experiments were conducted under USDA # 00-220-02n, 01-017-14n, 01-177-01n and 00-177-02n.

F.1. Greenhouse evaluations

F.1.a. Experimental methods – greenhouse study

Plant propagation and establishment

The greenhouse plants consisting of ASR368 R0 and F1 progeny evaluated for flowering characteristics in 2001 as described in Section VI.D.1 were evaluated for viable (based on germination test) seed set per seed head under open- and self- pollination conditions. In 2002, ASR368 F2 and the commercial cultivars Backspin, Crenshaw and Penn A-4 were evaluated for pollen size and viability in Section VI.E.1 were also evaluated for open-pollinated seed set.

The methods of floral initiation in the Iowa State University greenhouse were the same in both years and are detailed in Section VI.D.1.a of this petition.

Seed production and collection

Self-pollination. Just prior to anthesis in 2001, three inflorescences at similar developmental stages from each plant were pushed inside a hybridization bag (Lawson #411). The opening of the bag was then folded diagonally around the culms and secured with a jumbo paper clip at the bottom to isolate the panicles from cross-pollination and to prevent loss of seeds due to seed shattering. Bags were tapped lightly every day to facilitate pollen movement and self-pollination within the bag. Panicles were harvested approximately one month after bagging when seedheads were mature. Self-pollination studies were not performed in 2002.

Open-pollination. About two weeks after bagging panicles for self-pollination and when anthesis on the plants was complete, three additional randomly selected inflorescences

from each plant were bagged together in a fashion similar to that used for self-pollination in both 2001 and 2002.

Seeds were manually threshed out of panicles. The number of germinable seed per panicle were evaluated in 2001 whereas the number of mature seed per panicle were counted in 2002.

Germinable seeds per panicle, 2001. Seeds were pre-chilled at 5^0 C and were placed in a plastic Petri plate containing wet filter paper. After one week of pre-chilling, seeds were transferred to a six-inch plastic pot with 1/3 volume of fine sand and 2/3 volume of soilless medium for germination. Germination took place at 20 to 21^0 C. All pots were irrigated with 0.2% KNO_3 for one week and then irrigated to prevent moisture stress.

Seed set per panicle, 2002. The number of mature seeds from open-pollinated inflorescences were examined and recorded using a stereomicroscope when necessary. The criteria used for judging a seed being mature or not were seed color (dark brown for mature seeds) and grain fill (mature seeds appear plump).

Data collected

In 2001, seedling emergence was continuously monitored for one month and the number of seedlings that emerged were recorded. However, in 2002, fewer plants of ASR368 F2 and the commercial cultivars were evaluated for this experiment so they were pooled, as discussed in the previous section (VI.E.1).

Statistical analysis

As described in Section VI.D.1.a of this petition.

F.1.b. Results of greenhouse study

The mean number of seeds per three seed heads obtained from open-pollinated inflorescences (panicles) of the ASR368 genotypes, B99061R and the commercial cultivars in 2001 and 2002 are provided in Tables VI.F.1 and VI.F.3, respectively. The specific comparisons between the ASR368 genotypes with B99061R and the commercial cultivars for seeds set per three inflorescences in 2001 are provided in Table VI.F.2. The p-values ($\alpha = 0.05$) for 2001 indicate that the number of open-pollinated seed set by the ASR368 R0 and F1 plants was not significantly different from B99061R and the commercial cultivars, Penncross, Crenshaw and Penn A-4. In 2002, open-pollinated seed set of the ASR368 F2 plants was not significantly different from the combined conventional genotypes.

Self-pollinated seed set among all genotypes in 2001 was low, as expected, due to self-incompatibility systems known to exist in the *Agrostis* genus. Summary statistics are presented in Table VI.F.4 for 2001; however, no comparisons of the genotypes were performed on this data due to the limited and variable seed set observed. The maximum

viable seed set upon self-pollination occurred on the commercial cultivar, Crenshaw. The lowest viable seed set upon self-pollination occurred for both ASR368 R0 and B99061R. ASR368 F1 plants were intermediate to the three commercial cultivars.

Table VI.F.1. Number of observations, mean, standard deviation and the minimum and maximum values for the number of germinable seeds set on every three inflorescences (panicles) that were open pollinated among ASR368 R0 and F1 progeny B99061R and three commercial cultivars in 2001.

Genotype	Number of observations	Mean	Std. deviation	Minimum value	Maximum value
Penn A-4	10	29.83	24.10	5	73
Crenshaw	10	43.26	53.16	14	174
Penncross	9	23.42	20.00	3	59
B99061R	6	19.33	7.69	6	27
ASR368 R0	6	14.83	10.83	1	30
ASR368 F1	6	39.53	17.83	9	62

Table VI.F.2. Mean difference, standard error and the p value ($\alpha = 0.05$) associated with each contrast between ASR368 R0 or F1 progeny and B99061R and three commercial cultivars for number of seeds formed through open-pollination in 2001.

Contrast	Mean difference	StdErr	Probt
B99061R vs. ASR368 R0	19.33-14.83=4.50	21.45	0.84
B99061R vs. ASR368 F1	19.33-39.53=-20.2	19.97	0.33
PennA-4 vs. ASR368 R0	29.83-14.83=15.00	17.25	0.40
PennA-4 vs. ASR368 F1	29.83-39.53=-9.70	15.39	0.53
Crenshaw vs. ASR368 R0	43.26-14.83=28.43	17.48	0.12
Crenshaw vs. ASR368 F1	43.26-39.53=3.73	15.57	0.81
Penncross vs. ASR368 R0	23.42-14.83=8.59	17.46	0.63
Penncross vs. ASR368 F1	23.42-39.53=-16.11	15.55	0.31

Table VI.F.3. Number of observations, mean, standard deviation and the minimum and maximum values for the number of seeds set on every three inflorescences that were open-pollinated among creeping bentgrasses in 2002.

Group	Number of observations	Mean	Std deviation	Minimum value	Maximum value
Commercial cultivars	3	281.67	288.74	0	577.00
ASR368 F2	4	261.50	189.98	16	435.00
Pr > F ($\alpha = 0.05$)		0.99			

NS = no significant differences between groups detected by the F test ($\alpha = 0.05$).

Table VI.F.4. Number of observations, mean, standard deviation and the minimum and maximum values for the number of seeds set on every three inflorescences that were self-pollinated among creeping bentgrasses in 2001.

Genotype	Mean	Std. deviation	Minimum value	Maximum value
Penn A-4	0.78	1.09	0	3
Crenshaw	3.67	5.36	0	17
Penncross	1.67	2.50	0	7
B99061R	0.17	0.41	0	1
ASR368 R0	0.17	0.41	0	1
ASR368 F1	1.33	1.63	0	4

F.1.c. Conclusions for greenhouse evaluation of seed set

Based on the viable seed set results from both open- and self-pollination studies in the greenhouse in 2001 and 2002, it is not expected that that ASR368 plants would be different in their ability to cross- or self-fertilize than B99061R or commercially available commercial creeping bentgrass cultivars. Therefore, it is not expected that ASR368 would pose an additional pest risk based on these seed set characteristics than non-transgenic genotypes representative of *A. stolonifera.*

F.2. Field evaluations

Evaluations of the fecundity of ASR368 plants were performed under field conditions in both 2001 and 2002. In 2001, ASR368 R1 plants were evaluated in Franklin County, Washington at the two locations described in Section VI.D.2.a. In 2002, ASR368 F1 and F2 plants were evaluated in separate experiments in Jefferson County, Oregon as described in Section VI.D.2.a. For additional details of the methods of plant propagation and establishment of these experiments please refer to Section VI.D.2.a.

F.2.a. Experimental methods

Seed harvest

Seeds per Five Panicles (Seed heads): In both 2001 and 2002, panicles were first harvested from each plant at maturity by removing the flowering culm above the plant canopy when the majority of the panicles including the upper third of the culm were brown. Five mature random panicles were harvested from each genotype.

Gross Seed Weight and Clean Seed Weight: Panicles remaining on the plants after the five panicles were removed were harvested separately and processed to determine gross seed weight and clean seed weight. Seed was liberated from the panicles by rubbing panicles across a plastic screen drawn taut over a wooden frame measuring 24" by 24", which was placed over a plastic collection bin. Plant material passing through the initial screen and falling into the plastic container was collected. Collected material was then sifted through a 1/8" metal screen followed by a 1/17" and 1/21" metal screens. Seed was separated from the majority of plant material through sifting and captured in a solid bottomed tray underneath the screens and weighed to assess gross seed weight.

Vegetative Biomass: Remaining vegetative material was harvested from each plant at both locations of the trial to a height of 2" above the soil surface to use in determination of plant biomass (dry weight) and to avoid including soil in tissue samples.

Data collected

Gross Seed Weight: The partially clean seed from each plant collected, following the rubbing and screening procedures, was weighed to the nearest 0.1 g by gravimetric analysis to determine Gross Seed Weight.

Clean Seed Weight: In addition to the seed cleaning and screening described above, seed from the gross seed weight determination was separated from screenings by cleaning on a tabletop Clipper® seed cleaner with a single 1/20" round hole screen with air flow to purify the seed. The cleaner and screens were cleaned between each plant sample of seed. Clean seed for each individual plant was weighed to the nearest 0.1 g by gravimetric analysis.

Thousand Seed Weight: Clean seed from each individual plant was further purified on a General Blower (New Jersey Sheet Metal Works, NJ) set at an airflow setting of 8.0 for two minutes. One thousand seed from each purified sample were counted using a Pfeuffer Contador (Pfeuffer GmbH**,** Kitzingen, Germany) seed counter. Thousand seed weight for each clean seed sample was measured to the nearest 0.001 g by gravimetric analysis as an indirect index of seed size.

Seed Set per Five Panicles: Five panicles from each RR or RS plant in each location were individually hand-rubbed between the thumb and forefinger over a small metal pan. Seed were screened through a 1/17 and 1/20" screen and purified with a General Blower

as described for the 1000 seed weight. Pure seed were counted on a seed counter to determine seed set per five panicles through open pollination on each plant.

Statistical analysis

In 2001, vegetative biomass and the seed characteristics evaluated for the ASR368 R1 RR and RS were analyzed by pooling the data for each characteristic after having accounted for the potential variability contributed by location using Friedman's test (α = 0.05). In 2002, the data from the ASR368 F1 and F2 evaluations were analyzed separately using ANOVA (α = 0.05) to determine if any significant differences exist between the means of ASR368 and the collection of commercial cultivars. Fisher's LSD (α = 0.05) tests were run post-hoc if any ANOVA revealed the presence of significant differences.

F.2.b. Results of fecundity studies

2001

The results of the 2001 field study comparing vegetative biomass per plant, gross seed yield per plant, clean seed weight per plant and 1000 count seed weight evaluated for the ASR368 R1 RR and RS segregants are presented in Tables VI.F.5 through VI.F.9. The results for each characteristic at the first and second Franklin County, Washington sites were not significantly different according to Friedman's test (α = 0.05). Between the ASR368 R1 RR and RS progeny no statistically significant differences were identified for any of the seed productivity characteristics other than for vegetative biomass where RS plants had greater biomass than RR plants (Table VI.F.6). No impact on a greater ability to establish or persist is associated with this result since the biomass for the ASR368 R1 RS segregant was greater than that of the RR segregant.

Table VI.F.5. Number of seeds per five panicles for ASR368 R1 RR and RS segregants combined over the two Franklin County, Washington sites in 2001.

Number of Seeds per 5 Panicles							
Segregant	**n**	**Mean**	**Min**	**Max**	**SD**	**P-value**	
RR	66	2,282	132	4,880	887.2	**Site[1]**	**Segregants[2]**
RS	46	2,496	422	6,345	1,183.5	0.784	0.405

[1] Difference in the number of seeds per five seed heads for ASR368 R1 RR and RS segregants at both Franklin County, Washington locations was non-significant according to Friedman's test (α = 0.05).

[2] Difference between the number of seeds per five seed heads for ASR368 R1 RR and RS segregants was not significant according to Friedman's test (α = 0.05).

Table VI.F.6. Vegetative biomass (grams per plant) of ASR368 R1 RR and RS segregants combined over the two Franklin County, Washington sites in 2001.

Plant Biomass (g)							
Segregant	**n**	**Mean**	**Min**	**Max**	**SD**	**P-value**	
RR	64	163.0	40	413	96.3	**Site[1]**	**Segregants[2]**
RS	48	215.6	39	884	157.1	0.516	0.007

[1] Difference in the plant biomass of ASR368 R1 RR and RS segregants at both Franklin County, Washington locations was not significant according to Friedman's test ($\alpha = 0.05$).
[2] Difference in the plant biomass of ASR368 R1 RR and RS segregants was significant according to Friedman's test ($\alpha = 0.05$).

Table VI.F.7. Gross seed weight (grams per plant) of ASR368 R1 RR and RS segregants combined over the two Franklin County, Washington sites in 2001.

Gross Seed Weight (g/plant)							
Segregant	**n**	**Mean**	**Min**	**Max**	**SD**	**P-value**	
RR	65	9.5	0.2	41.5	8.9	**Site[1]**	**Segregants[2]**
RS	42	14.8	0.2	71.4	14.8	0.294	0.102

[1] Difference in ASR368 R1 RR and RS segregant seed weight at both Franklin County, Washington locations was not significant according to Friedman's test ($\alpha = 0.05$).
[2] Difference between ASR368 R1 RR and RS segregant seed weight was not significant according to Friedman's test ($\alpha = 0.05$).

Table VI.F.8. Clean seed weight (grams per plant) of ASR368 R1 RR and RS segregants combined over the two Franklin County, Washington sites in 2001.

Clean Seed Weight (g/plant)							
Segregant	**n**	**Mean**	**Min**	**Max**	**SD**	**P-value**	
RR	68	3.7	0.0	19.4	3.9	**Site[1]**	**Segregants[2]**
RS	47	5.3	0.1	30.3	6.5	0.642	0.715

[1] Difference in ASR368 R1 RR and RS segregant clean seed weight at both Franklin County, Washington locations was not significant according to Friedman's test ($\alpha = 0.05$).
[2] Difference between ASR368 R1 RR and RS segregant clean seed weight was not significant according to Friedman's test ($\alpha = 0.05$).

Table VI.F.9. One thousand seed weight (g) of ASR368 R1 RR and RS segregants combined over the two Franklin County, Washington sites in 2001.

1000 Count Seed Weight (g)							
Segregant	**n**	**Mean**	**Min**	**Max**	**SD**	**P-value**	
RR	63	0.073	0.040	0.140	0.015	**Site[1]**	**Segregants[2]**
RS	42	0.072	0.030	0.150	0.019	0.354	0.274

[1] Difference in ASR368 R1 RR and RS segregant 1000 seed weight at both Franklin County, Washington locations was not significant according to Friedman's test ($\alpha = 0.05$).

[2] Difference between ASR368 R1 RR and RS 1000 segregant seed weight was not significant according to Friedman's test ($\alpha = 0.05$).

2002

ASR368 F1 and F2 progeny were not significantly different from at least one of the commercial creeping bentgrass cultivars for characters including: seed set per five panicles, vegetative biomass per plant, gross seed weight per plant, clean seed weight per plant, and total seed count per plant.

The ASR368 F1 plants produced more seed per five panicles than Crenshaw and Penn A-4, but fewer seed than Backspin; however each of the numerical differences was not significant (Table VI.F.10). The ASR368 F2 produced significantly more seed per five panicles than Crenshaw and Penn A-4 but no significant difference was found compared to Backspin (Table VI.F.10). It is likely that any apparent increase in seed set per five panicles between ASR368 F1 and F2 progeny populations is due to the forward breeding process with ASR368 rather plant transformation since no significant differences were detected during the 2000-2001 field trials where seed set of RR and RS segregants from R1 generation seed were compared.

Vegetative biomass of ASR368 F1 and F2 progeny was not significantly different from that of Backspin (Table VI.F.11). Vegetative biomass of ASR368 F1 and F2 progeny was significantly less than that of Penn A-4. Crenshaw was not significantly different from the F1 progeny but produced significantly more biomass than ASR368 F2 progeny. Therefore, ASR368 F1 and F2 progeny are similar to commercial creeping bentgrass cultivars in biomass productivity and are unlikely to be more competitive or invasive due to increased growth potential.

ASR368 F1 and F2 progeny were not significantly different from at least one of the commercial cultivars for the characters including gross seed weight and clean seed weight (Tables VI.F.12 and VI.F.13). Variation among commercial cultivars for clean seed weight is apparent, but clean seed weight from ASR368 F1 and F2 progeny were intermediate to that of the commercial cultivars. Interestingly, the relative rank of ASR368 and cultivar populations for clean seed weight was similar in both the F1 and F2 comparative tests.

In 2002, the weight of 1000 clean seed (1000 count seed weight) of both ASR368 F1 and F2 progeny plants was significantly lower than each of the three commercial cultivars (Table VI.F.14). However, minimum 1000 count seed weight values for individual plants within Backspin and Crenshaw cultivars were similar to minimums observed for ASR368 progeny. Additionally, the 1000 count seed weight from ASR368 R1 progeny evaluated in 2001 was not significantly different from that of the R1 plants for this characteristic. Furthermore, a decrease in seed weight is a reflection of smaller seed size, which would not be expected to contribute to greater seedling establishment in unmanaged environments. Although some significant differences were identified between ASR368 F1 and F2 progeny and one or more commercial cultivars for the other seed characteristics, there did not appear to be a trend other than for smaller seed size.

Overall, ASR368 F1 progeny produced significantly fewer seed per plant than Backspin and were not significantly different from Crenshaw and Penn A-4 (Table VI.F.15). The ASR368 F2 progeny produced fewer total seed per plant than Backspin and Crenshaw but significantly more than Penn A-4. These results indicate that seed production is variable among the creeping bentgrass cultivars and that the ASR368 F1 and F2 progeny are within the range of the seed produced per plant of the commercial cultivars that are representative of *A. stolonifera.*

Table VI.F.10. Mean number of seed per five panicles for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Number of Seeds per Five panicles							Number of Seeds per Five panicles						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	34	1,245	152	2,847	661	1,014	1,475	40	1,841	92	3,804	997	1,522	2,160
Backspin	29	1,472	268	3,383	880	1,137	1,807	39	1,750	328	3,769	919	1,452	2,047
Crenshaw	35	1,161	331	2,947	661	934	1,388	38	1,316*	313	2,176	543	1,137	1,494
Penn A-4	27	1,078	50	2,347	636	826	1,330	37	911*	65	2,084	532	733	1,088
p value (α = 0.05)		0.26							<0.0001					
LSD (α = 0.05)		358.4							350.2					

* Means are significantly different from ASR368 F1 or F2, according to Fisher's LSD (α = 0.05)

Table VI.F.11. Vegetative biomass (grams per plant) of ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Plant biomass (g/plant)							Plant biomass (g/plant)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	37	265	127	486	81	238	292	40	320	102	715	147	273	367
Backspin	30	280	55	592	120	236	325	39	492	94	954	227	418	565
Crenshaw	35	313	123	534	122	271	355	38	569*	113	4,471	701	338	799
Penn A-4	27	540*	75	1,207	244	444	637	39	505*	143	1,359	278	415	595
p value (α = 0.05)	<0.0001							<0.0001						
LSD (α = 0.05)	72.84							177.37						

* Means are significantly different from ASR368 F1 or F2, according to Fisher's LSD (α = 0.05)

Table VI.F.12. Gross seed weight per plant (grams per plant) for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Gross seed weight/plant (g/plant)							Gross seed weight/plant (g/plant)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	37	105.5	30.8	184.6	37.0	93.1	117.8	40	149.4	45.2	317.4	62.7	129.4	169.5
Backspin	30	193.2*	34.2	362.4	77.7	164.2	222.2	37	142.4	27.0	410.0	86.5	113.6	171.3
Crenshaw	35	126.4	31.8	310.6	62.0	105.1	147.7	38	117.3	44.6	227.0	41.9	103.5	131.1
Penn_A-4	25	105.1	41.4	233.6	57.7	81.2	128.9	36	88.1*	5.6	313.4	62.5	67.0	109.2
p value (α = 0.05)	<0.0001							<0.0001						
LSD (α = 0.05)	29.42							29.54						

* Means are significantly different from ASR368 F1 or F2, according to Fisher's LSD (α = 0.05)

Table VI.F.13. Clean seed weight per plant (grams per plant) for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Clean seed weight/plant (g)							Clean seed weight/plant (g)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	36	8.3	0.01	24.00	6.5	6.1	10.5	40	12.9	1.20	34.00	8.9	10.1	15.7
Backspin	30	22.1*	0.40	58.80	14.8	16.6	27.6	37	17.3*	0.60	55.40	12.2	13.2	21.4
Crenshaw	35	13.2	0.80	46.80	10.7	9.5	16.9	38	14.7	2.00	29.80	7.1	12.3	17.0
Penn A-4	25	7.6	0.40	17.00	5.2	5.4	9.7	36	4.5*	0.06	25.00	4.9	2.8	6.1
p value (α = 0.05)	<0.0001							0.0						
LSD (α = 0.05)	4.98							3.94						

* Means are significantly different from ASR368 F1 or F2, according to Fisher's LSD (α = 0.05)

Table VI.F.14. One thousand seed weight (g) for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars in in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	1000 count seed weight (g)							1000 count seed weight (g)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	35	0.092	0.07	0.13	0.013	0.087	0.096	40	0.093	0.07	0.12	0.011	0.090	0.097
Backspin	30	0.101*	0.07	0.14	0.013	0.096	0.106	37	0.109*	0.08	0.14	0.013	0.104	0.113
Crenshaw	35	0.107*	0.07	0.14	0.018	0.101	0.113	38	0.108*	0.09	0.14	0.014	0.104	0.113
Penn A-4	25	0.131*	0.10	0.17	0.018	0.124	0.138	35	0.121*	0.09	0.16	0.019	0.114	0.127
p - value (α = 0.05)	<0.0001							<0.0001						
LSD (α = 0.05)	0.008							0.007						

* Means are significantly different from ASR368 F1 or F2, according to Fisher's LSD (α = 0.05)

Table VI.F.15. Seed count per plant[1] for ASR368 F1 and F2 progeny and commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Seed count/plant							Seed count/plant						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	35	90,765	6,667	242,500	65,307	68,331	113,199	40	138,019	13,333	377,778	92,823	108,333	167,706
Backspin	30	225,520*	3,636	588,000	151,873	168,809	282,230	37	161,901	6,000	554,000	115,016	123,552	200,249
Crenshaw	35	122,216	7,273	425,455	97,311	88,788	155,643	38	138,668	18,182	331,111	72,249	114,921	162,416
Penn A-4	25	58,866	3,333	141,667	41,367	41,790	75,941	35	38,554*	2,000	192,308	40,198	24,745	52,362
p value (α = 0.05)	<0.0001							<0.0001						
LSD (α = 0.05)	49,594							38640						

[1] Seed count per plant = [Clean seed weight per plant (g) / 1000 Count Seed Weight (g)] X 1000

* Means are significantly different from ASR368 F1 or F2, according to Fisher's LSD (α = 0.05)

F.3. Conclusions for fecundity studies

The purpose of these studies was to examine whether the insertion of the *cp4 epsps* gene into creeping bentgrass impacted the reproductive characteristics of ASR368 when compared to non-transgenic creeping bentgrass genotypes such that they might have a competitive advantage in managed or unmanaged ecosystems.

Greenhouse evaluations of open-and self-pollinated seed set for ASR368 R0 and F1 plants displayed no statistically significant differences from B99061R or the commercial cultivars, Penncross, Penn A-4 or Crenshaw. Self-pollinated seed set for the ASR368 genotypes was low, as expected, due to self-incompatibility systems known to exist in the *Agrostis* genus.

The 2001 field trial study at two locations resulted in no statistically significant differences between ASR368 RR and ASR368 RS half-sib progeny populations for each of the following characteristics: gross seed yield per plant, clean seed weight per plant, 1000 count seed weight and seed set per five panicles. The vegetative biomass of ASR368 R1 RR and RS progeny were significantly different. However, significant differences between the ASR368 F1 or F2 RR progeny and commercial cultivars in the 2002 studies were not consistent as Backspin was not significantly different from ASR368 F1 or F2 progeny populations. Nonetheless, reduced biomass would be inconsistent with an increase in weed potential.

The results of the 2002 ASR368 F1 and F2 trials demonstrated that ASR368 F1 and F2 RR progeny performance generally was within the range of values observed for commercial cultivars or was not significantly different from at least one of the commercial cultivars for characters including: seed set per five panicles, vegetative biomass per plant, gross seed weight per plant, clean seed weight per plant, and total seed count per plant. Seed of the ASR368 genotypes tended to be smaller than commercial cultivars as indicated by the 1000 count seed weight. However, there were individual conventional plants within Backspin and Crenshaw that had (minimum) 1000 count seed weights similar to the minimums observed from plants of ASR368 progeny. In addition, significant differences between commercial cultivars were evident as well and smaller seed is more likely to reduce establishment rate and competitive ability as seen for other grass species (Whalley *et al*., 1996). These results indicate that seed production is variable among the creeping bentgrass cultivars and that the ASR368 F1 and F2 are within the range of the seed produced per plant by these commercial cultivars that are representative of *A. stolonifera.*

Considering the 2001 and 2002 results of the fecundity studies, which encompassed three generations of ASR368, it is not expected that ASR368 varieties would differ in their ability to produce seed compared to commercial cultivars. This supports a conclusion of no contribution to increased weed potential based on the seed characteristics of ASR368 compared to commercial creeping bentgrass cultivars that are representative of *A. stolonifera.*

G. Seed Physiology

Seed viability, seedling vigor, dormancy and longevity are important characteristics to assess whether ASR368 seed is different from that of existing creeping bentgrass cultivars. If these seed physiological characteristics have declined, varieties developed with ASR368 may be less desirable to seed producers or golf course superintendents. However, if these same characteristics are somehow enhanced, there is the potential that ASR368 may be more invasive or persistent in the environment. Although seed persistence data from controlled laboratory or field research is not common, there are recognized tests and modifications of them useful to assess seed viability, seedling vigor, dormancy and longevity. These include: (1) standard germination test (SGT); (2) sub-optimal temperature (SUB); (3) supra-optimal temperature (SuOP) and (4) an accelerated aging test (AAT).

These four tests were used to compare the relative seed and seedling performance of ASR368 R1 RR and RS segregants and two commercial bentgrass varieties, SR 1020 and Highland bentgrass (*A. castellana*).

AOSA Standard Germination Test

Seed viability was evaluated by the SGT as described in the Association of Official Seed Analysts Rules for Testing Seeds (1998). The SGT provides an ideal germination condition to obtain the maximum potential germination of the seed lot.

Total germination percentage (% viability) was used as an indicator of seed-lot quality and to provide a baseline for the ratio of RR to RS progeny to expect under ideal conditions. The percentage viability of each genotype in the SGT was also used as a baseline for comparing the percentage of the same seed germinating under the conditions of the SUB, SuOP and AAT tests.

Germination Rate (GR)

Germination rate or the speed of germination is determined by using the results of the SGT. The GR is considered one index of seed vigor and germination energy (Kulik and Yaklich, 1982; Tekrony and Elgi, 1977). Seeds with similar total germination percentage vary in their rate of germination and growth (i.e., speed of germination). Vigorous seeds germinate faster than medium or poor quality seeds. Germination rate calculations for each genotype were compared to determine if the seedlots were of similar vigor. Germination rate does not test for unintended effects of the *cp4 epsps* gene since the R1 seedlot is expected to segregate 1:1 for RR and RS phenotypes.

Sub-Optimal Temperature (SUB) and Supra-Optimal Temperature (SuOP) Germination Tests:

Vigorous seeds germinate under a wide range of extreme temperatures. Beard (1973) describes the optimum temperature range as 15 to 24°C for cool season turfgrass growth.

Therefore, the objective of these two stress tests was to evaluate the germination and development of seedlings of each genotype under continuous sub- and supra-optimal temperatures (14ºC and 32ºC, respectively). If the ratio of RR to RS changes significantly under varying environmental stresses (SUB and SuOP) compared to the ratio under SGT, then it could be concluded that the transgene imparts a selective advantage for germination under stress.

Accelerated Aging Test (AAT)

The AAT can be used as an indication of the potential relative longevity of seeds or as an indication of relative seed survivability or longevity in the soil (Delouche and Baskin, 1973). The test exposes the seeds to high temperature and high relative humidity (45ºC and near 100% relative humidity) for 30 hours. The decline in germination following this period of stress is proportional to the level of seed vigor and its potential physiological longevity.

G.1. Experimental methods

G.1.a. General test conditions

Each of the four germination tests was conducted with four replications each of 100 seeds of the three test genotypes; ASR368 R1, SR1020 and Highland bentgrass. Germination percentage was recorded each week for four weeks. Seedlings emerging during each weekly evaluation were marked with different colored pins and beads for later classification of germination rate (SGT only) or relative germination energy and seedling vigor. Seedlings were evaluated according to the AOSA Seedling Evaluation Handbook, 1992.

At the end of each test period (28 days), plants were moved to a greenhouse at 24°C ± 6°C and continuous light. Uniform nutrition and continuous irrigation were provided to all plants to prevent visible nutrient deficiencies and drought stress. Plants of each ASR368 R1 seed lot were sprayed with Roundup herbicide approximately two weeks after moving them to the greenhouse when the first 50% of all seedlings had reached the first tiller stage to determine the percentage of RR and RS seedlings.

G.1.b. Specific germination test conditions

Standard Germination Test (SGT)

Seed samples were evaluated for viability using the standard germination test as described in the AOSA Rules for Testing Seeds, 1998. Four replications of 100 seeds each were pre-chilled at 10°C for five days before moving to15/25°C for the 28-day test period. Seeds of each line were germinated in a growth chamber under alternating 15°C in the dark and 25°C in lighted conditions. Supplemental light with an intensity of 125 ft-c was provided by a cool white fluorescent source.

Germination Rate (GR)

Seedlings that germinated during each seven-day period during the four weeks of the SGT test were marked with different colored pins for easier identification. Germination counts from the standard germination test reported at each seven-day interval for 28 days were used to calculate the germination rate.

The following equation was used to calculate the rate (speed) of germination, GR;

$$GR = \frac{number\ of\ normal\ seedlings}{days\ of\ \ first\ count} + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots + \frac{number\ of\ normal\ seedlings}{days\ of\ \ final\ count}$$

Sub- and Supra-Optimal Temperature Tests (SUB and SuOP)

In the sub-optimal temperature germination test, seeds of each seed lot were germinated under constant 14º C for four weeks before moving them to the greenhouse for seedling vigor determinations. The test can identify if RR seeds are more likely to germinate than RS seed under sub-optimal temperatures. In the supra-optimal temperature germination test, seeds of each event or cultivar were germinated under constant 32º C for four weeks before moving seedlings to the greenhouse for seedling vigor determinations. The SuOP test can identify if RR seeds are more likely to germinate than RS seeds under supra optimal temperatures. High-pressure sodium bulbs provided supplemental light.

Accelerated Aging Test (AAT)

Seeds of each seed lot were exposed to 45ºC and near 100% relative humidity for 30 hours. The decline in germination following this stress is proportional to the level of seed vigor and its potential physiological longevity or dormancy. The test was performed using the wire-tray mesh method described by McDonald and Phaneendranath (1978) in the AOSA Seed Vigor Testing Handbook, 1983. Following AAT, seed were tested for viability under standard AOSA temperature conditions for 28 days. The AAT test can determine if RR seed are more likely to persist than RS seed.

G.1.c. Germination energy and seedling vigor

During the first two weeks in the greenhouse following each of the four test procedures, seedlings of each genotype within each test were marked and divided into sub-populations based on their relative germination energy (date of germination) and seedling vigor. The first 50% of the plants that reached the first tiller stage among all seedlings from the seven-day germination count were classified as having high germination energy and seedling vigor. Slower developing seedlings remaining from the seven-day count along with the seedlings germinating after the 14, 21 and 28-day counts, were classified as having low germination energy and seedling vigor.

Following the two-week greenhouse acclimation and the classification into high or low germination energy and seedling vigor, sub-populations of the ASR368 R1 plants were sprayed with a 50:50 mixture of Roundup Pro and Roundup Accord herbicides (total rate of 1 gal/acre) in a greenhouse spray chamber. Percentage RR was determined for each sub-population two weeks after spraying with glyphosate.

G.1.d. Data collected

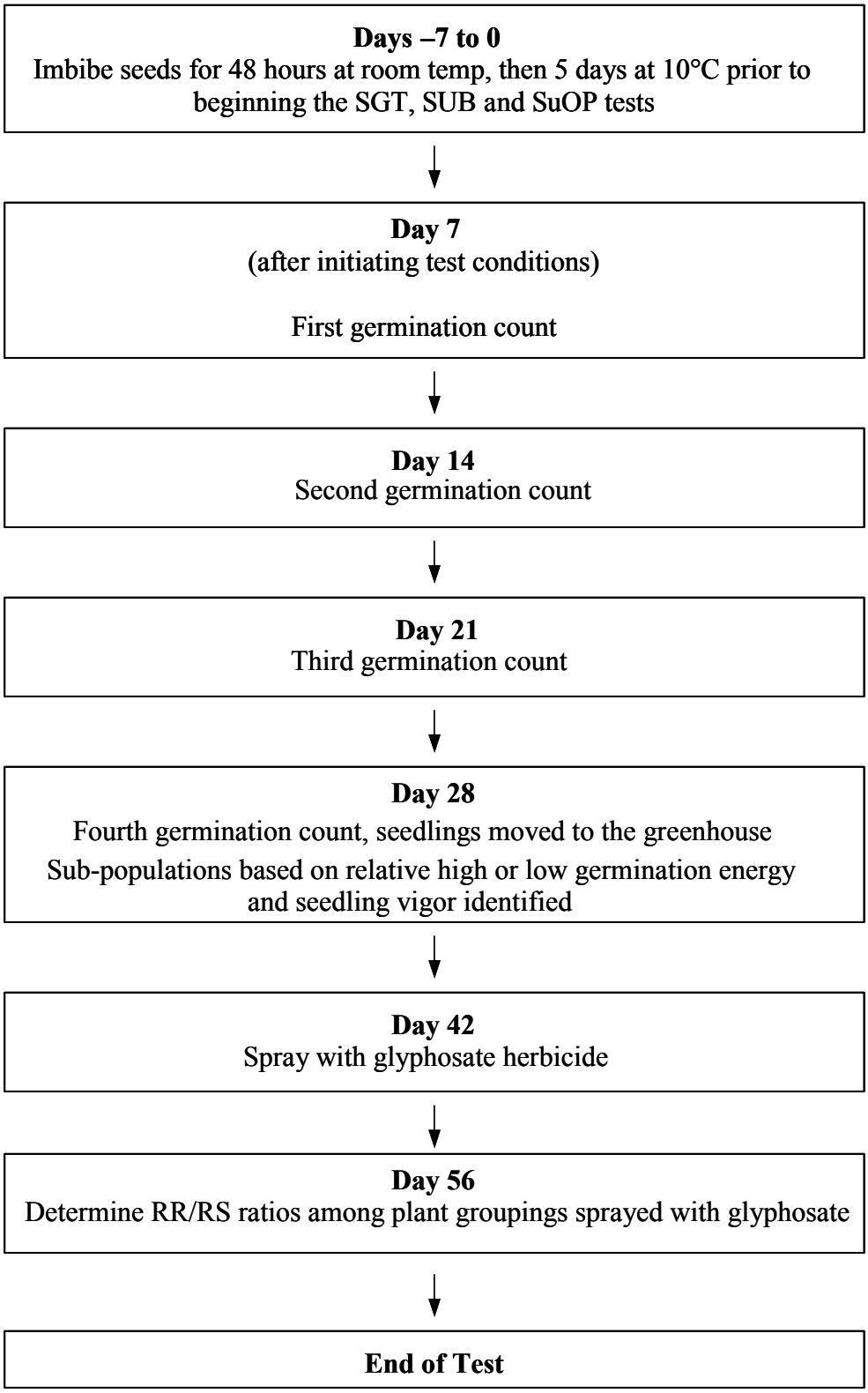
Data for the different experiments included in the study were collected according to Figure VI.G.1.

G.2. Data analysis

ASR368 R1 seed was expected to segregate 1:1 for Roundup tolerant (RR) and Roundup sensitive (RS) phenotypes. Consequently, it was possible to compare ASR368 RR and RS phenotypes in each experiment. Potential gene effects on seed viability or longevity under stress were evaluated by comparing the baseline percentage of RR progeny recovered from SGT (optimum germination conditions) to the percentage of RR progeny recovered from stress environments, SUB, SuOP or AAT. If there were an increase in the percentage of RR progeny among seedlings that germinate during a stress test compared to that found during the SGT, increased relative stress tolerance of ASR368 R1 RR seed could be indicated. Duncan's Multiple Range Test was used to compare the mean percentage RR recovered from SGT to that recovered from other stress tests.

As described above, seedlings that germinated during each test were further divided into sub-populations based on relative germination energy and seedling vigor. Seedlings with high germination energy and seedling vigor were identified as the first 50% of seedlings to develop a new tiller among all seedlings that germinated within the first seven days of the test. Seedlings with low germination energy and seedling vigor included all remaining seedlings from the seven-day count plus all of those that germinated by the end of the test (28 days). Potential gene effects on germination energy and seedling vigor were evaluated by comparing the percentage of RR in sub-populations categorized by high germination and seedling vigor vs. low germination energy and seedling vigor within each of the seed tests. A t-test was used to compare means between sub-populations within each test environment.

Figure VI.G.1. Method of collecting data for the SGT, SUB, SuOP, and AAT seed physiology tests.



G.3. Results

G.3.a. AOSA Standard Germination Test (SGT)

The percentage germination results under the standard AOSA germination test showed no statistically significant difference for seed viability between seed lots derived from ASR368, SR1020 and Highland (Table VI.G.1). The germination percentages for these cultivars were 87.8, 89.8, and 94.5, respectively. Moreover, the viability of the ASR368 R1 seed lots met standards acceptable for certified creeping bentgrass seed (i.e., >85% germination).

Table VI.G.1. Percentage germination of ASR368 R1 seed segregating for RR and RS progeny, and two commercial cultivars, SR 1020 and Highland, following four seed quality tests.

Genotype	Germination (%)			
	SGT[2]	SUB[3]	SuOP[4]	AAT[5]
ASR368	87.8 bcd[1]	87.0 cde	81.5 de	88.5 abc
SR 1020	89.8 abc	93.5 abc	90.3 abc	73.3 f
Highland	94.5 ab	91.5 abc	80.8 e	90.3 abc

[1] Means followed by the same letter are not significantly different at P > 0.05according to Duncan's Multiple Range Test.
[2] Standard Germination Test
[3] Sub-optimal test at 14°C
[4] Supra-optimal test at 32°C
[5] Accelerated aging test at 45°C for 30h, final germination count was completed after five weeks.

The total percentage of RR plants among ASR368 R1 seedlings was 49.23% (173 RR of 351 total plants, Table VI.G.2), which is consistent with the approximate expected 1:1 segregation of RR to RS within the R1 seed lots produced from the hemizygous R0 primary transformant of event ASR368 (see Figure V.14).

Table VI.G.2. Percentage of ASR368 RR progeny recovered from segregating ASR368 R1 seed following four seed quality tests.

Genotype	Mean % RR plants			
	SGT[2]	SUB[3]	AAT[4]	SuOP[5]
ASR368	49.23a[1]	51.10a	50.28a	39.53b

[1] Means followed by the same letter are not significantly different at $\alpha = 0.05$ according to Duncan's Multiple Range Test.
[2] Standard Germination Test
[3] Sub-optimal temperature germination test at constant 14°C
[4] Accelerated aging test at 45°C for 30h
[5] Supra-optimal temperature germination test at constant 32°C

The percentage of RR plants among seedlings with high germination energy and seedling vigor was not significantly different (α = 0.05) from the percentage of RR plants among seedlings with lower germination energy and seedling vigor within ASR368 R1 (Table VI.G.3). Therefore, the *cp4 epsps* gene, its insertion and production of the CP4 EPSPS protein in ASR368 have no impact on germination energy or seedling vigor. Seed derived from ASR368 is no more likely to germinate or establish faster or be more competitive or invasive than seed that does not possess the Roundup Ready trait.

Table VI.G.3. Comparison of the percentage of ASR368 RR recovered within two sub-populations of ASR368 R1 seedlings as characterized by high and low relative germination energy and seedling vigor following four seed quality tests.

	Mean % RR Plants				
Seed Quality Test	**High Vigor[1]**	**S.D.**	**Low Vigor[2]**	**S.D.**	**Probt[3]**
SGT	49.7	0.77	49.05	3.79	0.78 ns
SUB	52.45	5.19	50.52	13.74	0.85 ns
SuOP	47.13	3.29	32.48	12.09	0.08 ns
AAT	50.00	5.80	50.45	4.09	0.92 ns

[1] % RR within the 50% of the plants that germinated by seven days and were first to reach the first tiller stage.
[2] % RR within plants that germinated and reached the first tiller stage later than those of the early developed plants.
[3] ns = not significant at α = 0.05

G.3.b. Germination rate

The germination rate of ASR368 (GR = 24.53) was not significantly different from that of SR 1020 (GR = 25.40) (Table VI.G.4). However, both seed lots had a lower germination rate compared to Highland (GR = 27.95). These results suggest that the overall vigor or germination energy of the ASR368 R1 seed was not different from commercial creeping bentgrass seed.

Table VI.G.4. Germination rate of ASR368, R1 seed segregating for RR and RS progeny and two commercial cultivars, SR 1020 and Highland.

	Percent Germination								
Genotype	**After 1 week**				**After 4 weeks**				**Germination rate[1]**
	Mean	**Min**	**Max**	**SD**	**Mean**	**Min**	**Max**	**SD**	
ASR368	79	74	84	4.2	88	86	91	2.4	24.53
SR 1020	82	73	88	6.3	90	84	95	4.8	25.40
Highland	94	92	96	1.7	95	93	96	1.3	27.95
LSD (0.05)	8.5				4.63				1.86

[1] Germination rate was calculated according to the AOSA Seed Vigor Testing Handbook (1998) and is an indication of the speed of germination of each genotype. It may also be used as an index of seed vigor and germination energy, whereby seed germination, germination energy and seed vigor increase as the index value increases.

G.3.c. Sub-optimal temperature test

Germination percentage of ASR368 R1 seed did not differ significantly from that of the commercial bentgrass cultivars, SR 1020 and Highland during the sub-optimal temperature (14°C) test (Table VI.G.1).

The percentage of ASR368 RR seedlings recovered under the SUB test was not significantly different from the respective percentage of RR seedlings recovered under standard AOSA conditions (SGT) (Table VI.G.2). Therefore, it can be concluded that ASR368 seed will not germinate and establish differently under sub-optimal temperatures.

The percentage of ASR368 RR seedlings with high germination energy and seedling vigor (52.45%) was not significantly different ($p > 0.05$) from the percentage of RR seedlings with lower germination energy and seedling vigor (50.52%). Consequently, seeds and seedlings of ASR368 will not be expected to have different germination energy and vigor characteristics under low temperature stress than commercial bentgrass cultivars.

G.3.d. Supra-optimal temperature test

Under the high temperature (32ºC) stress conditions of the SuOP test the germination of ASR368 RR seed was not different from that of Highland bentgrass with 81.5 and 80.8% rates, respectively (Table VI.G.1). However, in the same test, the germination percentage of SR 1020 was significantly greater than both ASR368 RR and Highland.

Mean germination (viability) of ASR368 RR seed under conditions of SuOP was not significantly different from the germination rates observed under standard AOSA conditions (Table VI.G.1). However, the percentage of ASR368 RR progeny recovered

following SuOP conditions was significantly lower than that recovered from standard AOSA conditions (Table VI.G.2).

Results of the SuOP test indicate that the percentage of ASR368 RR among seedlings with high germination energy and seedling vigor was not significantly different ($p > 0.05$) from the percentage of ASR368 among seedlings with lower germination energy and seedling vigor (Table VI.G.3). Therefore, supra-optimal temperatures do not appear to impact the germination energy and seedling vigor of ASR368 seedlings.

The poor germination of ASR368 RR seed during the SuOP test relative to the other seed tests may be attributed to the constant heat stress (32°C for 28 days) the young seedlings were exposed to in this study. Heat stress may have weakened seedlings and predisposed both the RR and RS plants to the post-SuOP test Roundup treatments at high rates. Beard (1973) describes the optimum temperature range for cool season turfgrass growth as 15 to 24°C. DiPaola and Beard (1992) also found that creeping bentgrass has a lower threshold for heat killing temperature and a lower time exposure threshold than several other cool season grasses. As a result, the ASR368 RR plants expected to survive following the application of glyphosate may have died due to heat stress, which ultimately decreased the potential percentage of RR seedlings and consequently, the apparent percentage of RS. RS seedlings that were expected to die following treatment with glyphosate may have died from both heat stress and the glyphosate treatment.

The higher percentage of ASR368 RR seedlings (47.13%) among high germination energy plants than among the low germination energy plants in this test suggests increased overall mortality from heat stress among the weak seedlings that germinated later (Table VI.G.3). The percentage of RR seedlings within the high germination energy sub-population is very similar although lower as compared to the overall percentage of RR seedlings (49.23 – 51.10%) observed under each of the other test environments (Table VI.G.2).

G.3.e. Accelerated Aging Test (AAT)

The stress conditions of the accelerated aging test (i.e., 45°C for 30 h at relative humidity of approximately 100%) reduced the percentage of seed germinating as well as the growth rate of seedlings throughout the test (Table VI.G.5). Seedlings of all genotypes were smaller compared to the standard germination test. However, by the end of the test period, SR 1020 had significantly lower germination than either ASR368 RR and Highland (Tables VI.G.1 and VI.G.5). Seed longevity in the AAT was likely dependent upon the physiological condition of the seed at the time the test was performed. The work of Elias and Copeland (1997) support this conclusion. These authors reported that the tolerance of multiple lots of canola seed to the conditions of accelerated aging was a reflection of their physiological quality at the time they were tested.

Table VI.G.5. Seed longevity of ASR368 R1 seed segregating for RR and RS progeny, and two commercial cultivars, SR 1020 and Highland as measured by percentage germination following the Accelerated Aging Test[1].

	Percent Germination[2]											
Genotype	**After 1 week**				**After 2 weeks**				**After 5 weeks**			
	Mean	**Min**	**Max**	**SD**	**Mean**	**Min**	**Max**	**SD**	**Mean**	**Min**	**Max**	**SD**
ASR368	19b[3]	14	28	6.4	75b	65	84	8.0	89a	86	91	1.1
SR 1020	28b	14	43	11.9	59c	40	76	14.7	70b	62	79	7.3
Highland	61a	55	63	3.7	88ab	85	91	2.5	90a	87	92	2.1

[1] Seeds were exposed to 45°C for 30 hours at relative humidity of near 100% before germinating the seeds. The accelerated aging test is used to predict the potential longevity of seeds.

[2] Slower growth rate of all genotypes was observed as a result of the stress conditions of the test compared to non-stressed seeds in the standard germination test.

[3] Means followed by the same letter in each column are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

The total percentage of RR seedlings from ASR368 R1 seed that germinated following exposure to the conditions of the AAT was not different from the respective percentages of ASR368 R1 RR seedlings from seed germinating under the SGT conditions (Table VI.G.2).

The percentage of RR seedlings with high germination energy and seedling vigor was not different from the percentage of RR seedlings with lower germination energy and seedling vigor within ASR368 R1 progeny following the AAT test. Therefore, seed or seedlings of ASR368 would not be expected to have greater longevity, germination energy or seedling vigor than other bentgrasses.

G.3.f. Germination energy and seedling vigor

The percentages of RR plants identified within the sub-populations of ASR368 R1 seedlings characterized by either high or low germination energy and seedling vigor were not significantly different following any of the four germination tests (Table VI.G.3). These results indicate that seed of the RR segregants from ASR368 R1 do not exhibit novel germination or developmental characteristics compared to RS segregants from the same R1 seed lot and seedling population. Therefore, seed or seedlings of ASR368 would not be expected to be more persistent, vigorous or invasive under diverse environmental conditions or stresses.

G.4. Overall conclusion for seed physiology (viability, vigor, dormancy and longevity)

The total germination percentage of ASR368 R1 seed during the SGT was not significantly different from those obtained during the SuOP, SUB and AAT tests (Table

VI.G.1). The percentage of RR progeny recovered from seedlings that germinated during the SGT was not significantly different from the percentage of RR observed during both the SUB and AAT. Therefore, ASR368 seed and seedlings would not be expected to germinate and survive under suboptimal temperatures or to persist longer than those of commercial bentgrass cultivars.

The viability and longevity of ASR368 R1 RR and RS seed were not different across germination environments as indicated by the percentage of RR seedlings identified following the SGT, AAT and SUB tests. However, in the SuOP test, a reduction in the apparent viability of ASR368 RR seed was attributed to the constant heat stress the seedlings were exposed to in this test. Heat stress may have predisposed both the ASR368 RR and RS plants to increased mortality regardless of the post-test glyphosate treatments. Nonetheless, the apparent reduction in survival of ASR368 RR seedlings under conditions of the SuOP test would not contribute to increased invasiveness or persistence of Roundup Ready bentgrass.

Germination energy and seedling vigor (establishment) of ASR368 RR and RS seedlings were not different across germination environments as indicated by the percentages of RR seedlings with high or low germination energy and seedling vigor.

In summary, the data from these diverse germination studies provides sufficient evidence to support the conclusion that seed and seedlings of ASR368 would not be expected to demonstrate greater survival, longevity, dormancy or vigor in diverse environments than commercial bentgrass cultivars.

H. Botanical Structures

A number of botanical structures are important characteristics when taxonomically identifying a plant as *A. stolonifera.* These characteristics help establish familiarity with the species, which can be used as a means of comparing plants of ASR368 with other commercial creeping bentgrass cultivars. Familiarity is in essence a baseline or the expected variability common to plants of the same species for a particular plant characteristic.

In this section, a number of vegetative and floral structures of ASR368 F1 and F2 plant populations, Elite Parent Plants (EPPs) and commercial cultivars were measured in the field and greenhouse in 2001 and 2002 (USDA # 00-220-02n, 01-017-14n, 01-177-01n and 00-177-02n). The characteristics evaluated included: the presence or absence of stolons, nodes, flag leaf, floral panicles, ligules, prominent venation on the abaxial leaf surface and vernation of the bud leaf. These features are characteristic in the taxonomic diagnosis of creeping bentgrass and may also contribute to the dispersal and invasiveness of a creeping bentgrass plant.

In addition, in 2001 and 2002, data on the mean number of florets per inflorescence and inflorescence (panicle) length (2002 only) were taken from plants grown in the Iowa State University greenhouse study discussed in Section VI.D – VI.F.

H.1. Experimental methods - field studies

Field studies were established in October 2000 in Franklin County, Washington and Jefferson County, Oregon and in September 2001 in Jefferson County, Oregon. The botanical characteristics of these plants were evaluated in 2001 and 2002, respectively.

Creeping bentgrass genotypes

In 2001, the botanical characteristics of ASR368 R1 progeny (RR) and Elite Parent Plants (RS) were evaluated. The botanical characteristics of ASR368 F1 and F2 plants were evaluated in separate experiments in 2002 at two locations in Jefferson County, Oregon as described above in Section VI.F.2. Three commercial creeping bentgrass cultivars were used as comparators in the 2002 experiments.

Plant propagation and establishment

2001 - Study. Seed of the above genotypes was germinated in a poly-house in Marion County, Oregon. Two weeks after germination, the F1 seedlings were sprayed with Roundup Pro herbicide to remove the RS segregates and surviving RR plants were transplanted to Jiffy plugs. Upon growth to the two to three tiller stage, the plantlets were transferred to the field sites in Jefferson County, Oregon on October 6, 2000 and Franklin County, Washington on October 17, 2000. Upon arrival at the field sites the plants were immediately space-planted in rows with seven plants per row. Elite Parent Plants (RS) were planted in alternating rows with the ASR368 F1 (RR) progeny.

All plants were irrigated as needed to prevent plant stress and fertilized to maintain good plant vigor for seed production and to avoid visual nutrient deficiencies. Weeds were controlled through both post emergent herbicide applications and hand weed control cultivation.

2002 - Study. Botanical characteristics were evaluated on the same plants from which flowering and fecundity data were taken for section VI.D.2. The methods used to propagate and establish the plants in these experiments are described in Section VI.D.2.a.

Sample Collection

In both 2001 and 2002, tillers with panicles in late anthesis were randomly harvested at the second node subtending the inflorescence. All samples were air dried in a laboratory prior to botanical evaluations.

Data collected

All botanical and morphological observations were conducted and recorded on the dried plant material in an enclosed laboratory in 2001 and 2002. Observed and recorded botanical and morphological characters included: presence of panicle and panicle length, presence or absence of anthers, stolons with nodes, flag leaf length and width, flag leaf sheaf length, ligule length, venation of abaxial leaf blade surface, and vernation of bud shoots. In addition, the number of florets per panicle was recorded in 2001.

Statistical analysis

In 2001, the botanical characteristics of the ASR368 F1 (RR) and EPP (RS) were analyzed by pooling the data for each characteristic after having accounted for the potential variability contributed by location using Friedman's test ($\alpha = 0.05$). In 2002, the data from the ASR368 F1 and F2 evaluations were analyzed separately using ANOVA ($\alpha = 0.05$) to determine if any significant differences exist between the means of ASR368 and the collection of commercial cultivars. Fisher's LSD ($\alpha = 0.05$) tests were run post-hoc if any ANOVA revealed the presence of significant differences.

H.2. Results – field studies

2001 Results

The mean and p-value ($\alpha = 0.05$) for the ASR368 RR and EPP (RS) populations combined across sites for the botanical characteristics evaluated in 2001 are presented in Tables VI.H.1 through VI.H.6. The analysis revealed no significant differences between the ASR368 RR and RS populations for each of the characteristics with the exception of ligule length. However, ligule length observed for both RR and RS phenotypes is within the range of values attributed to *A. stolonifera* in published botanical manuals (Hitchcock,

1951). Nonetheless, all botanical features were observed to exist as a normal plant character in both the RR and RS populations at both locations.

Table VI.H.1. Panicle length (cm) among ASR368 F1 RR and RS EPP plants evaluated in Jefferson County, Oregon and Franklin County, Washington in 2001.

Panicle Length (cm)	n	Mean	Min	Max	SD	P-value	
RR	51	9.20	5.80	11.80	1.63	**Site[1]**	**RR/RS[2]**
RS	37	9.50	6.00	14.10	2.21	0.103	0.237

[1] Difference in ASR368 R1 RR and RS EPP panicle length at Franklin County, Washington and Jefferson County, OR locations was not significant according to Friedman's test ($\alpha = 0.05$).
[2] Difference between ASR368 R1 RR and RS EPP panicle length was not significant according to Friedman's test ($\alpha = 0.05$).

Table VI.H.2. Flag leaf length (cm) among ASR368 F1 RR and RS EPP plants evaluated in Jefferson County, Oregon and Franklin County, Washington in 2001.

Flag Leaf Length (cm)	n	Mean	Min	Max	SD	P-value	
RR	50	6.17	2.50	9.60	1.63	**Site[1]**	**RR/RS[2]**
RS	36	5.66	2.00	9.30	1.96	0.201	0.419

[1] Difference in ASR368 R1 RR and RS EPP flag leaf length at Franklin County, Washington and Jefferson County, OR locations was not significant according to Friedman's test ($\alpha = 0.05$).
[2] Difference between ASR368 R1 RR and RS EPP flag leaf length was not significant according to Friedman's test ($\alpha = 0.05$).

Table VI.H.3. Flag leaf width (cm) among ASR368 F1 RR and RS EPP plants evaluated in Jefferson County, Oregon and Franklin County, Washington in 2001.

Flag Leaf Width (cm)	n	Mean	Min	Max	SD	P-value	
RR	50	0.31	0.10	0.50	0.08	**Site[1]**	**RR/RS[2]**
RS	36	0.29	0.10	0.50	0.10	0.115	0.562

[1] Difference in ASR368 R1 RR and RS EPP flag leaf width at Franklin County, Washington and Jefferson County, OR locations was not significant according to Friedman's test ($\alpha = 0.05$).
[2] Difference between ASR368 R1 RR and RS EPP flag leaf width was not significant according to Friedman's test ($\alpha = 0.05$).

Table VI.H.4. Flag leaf sheath length (cm) among ASR368 F1 RR and RS EPP plants evaluated in Jefferson County, Oregon and Franklin County, Washington in 2001.

Flag Leaf Sheath Length (cm)	n	Mean	Min	Max	SD	P-value	
RR	51	8.18	5.00	10.50	1.27	**Site[1]**	**RR/RS[2]**
RS	36	8.59	6.00	12.20	1.74	0.059	0.098

[1] Difference in ASR368 R1 RR and RS EPP flag leaf sheath length at Franklin County, Washington and Jefferson County, OR locations was not significant according to Friedman's test ($\alpha = 0.05$).

[2] Difference between ASR368 R1 RR and RS EPP flag leaf sheath length was not significant according to Friedman's test ($\alpha = 0.05$).

Table VI.H.5. Ligule length (cm) among ASR368 F1 RR and RS EPP plants evaluated in Jefferson County, Oregon and Franklin County, Washington in 2001.

Ligule Length (cm)	n	Mean	Min	Max	SD	P-value	
RR	50	0.24	0.10	0.35	0.05	**Site[1]**	**RR/RS[2]**
RS	36	0.27	0.10	0.40	0.08	0.034	0.003

[1] Difference in ASR368 R1 RR and RS EPP ligule length at Franklin County, Washington and Jefferson County, OR locations was significant according to Friedman's test ($\alpha = 0.05$).

[2] Difference between ASR368 R1 RR and RS EPP ligule length was significant according to Friedman's test ($\alpha = 0.05$).

Table VI.H.6. Number of florets per panicle among ASR368 F1 RR and RS EPP plants evaluated in Jefferson County, Oregon and Franklin County, Washington in 2001.

Number Florets per Panicle	n	Mean	Min	Max	SD	P-value	
RR	51	443	185	852	154	**Site[1]**	**RR/RS[2]**
RS	37	497	146	764	148	0.119	0.391

[1] Difference in ASR368 R1 RR and RS EPP number of florets per panicle at Franklin County, Washington and Jefferson County, OR locations was not significant according to Friedman's test ($\alpha = 0.05$).

[2] Difference between ASR368 R1 RR and RS EPP number of florets per panicle was not significant according to Friedman's test ($\alpha = 0.05$).

2002 Results

The mean, p–value (α = 0.05) and LSD (α = 0.05) for the ASR368 F1 and F2 comparisons to the commercial cultivars in 2002 are presented in Tables VI.H.7 through VI.H.11. The ASR368 F1 was not significantly different from the commercial cultivars for any of the botanical characteristics with the exception of panicle length. The panicle length of the ASR368 F1 plants was significantly shorter than the commercial cultivars. However, the panicle lengths of both RR and RS phenotypes are within the range of those published in botanical manuals (Hitchcock, 1951). The flag leaf width of ASR368 F2 was significantly narrower than Backspin and Penn A-4, flag leaf sheath length was significantly longer than Crenshaw and Penn A-4 and ligule length was significantly shorter than Backspin. Despite these differences, all of the botanical features evaluated were observed to exist as a normal plant character in both the ASR368 F1 and F2 populations during 2002.

Table VI.H.7. Panicle length (cm) of ASR368 F1 and F2 progeny and three commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Panicle length (cm)							Panicle length (cm)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	38	8.14	6.33	10.75	1.04	7.80	8.48	40	8.64	4.50	11.25	1.47	8.17	9.10
Backspin	28	9.37*	6.67	13.00	1.58	8.76	9.98	37	8.34	5.83	11.50	1.52	7.83	8.85
Crenshaw	34	9.76*	6.67	13.50	1.82	9.13	10.40	38	8.95	6.17	12.17	1.41	8.48	9.41
Penn A-4	26	9.33*	5.83	13.00	1.73	8.63	10.03	38	8.25	6.00	13.50	1.46	7.78	8.73
p value (α = 0.05)	<0.0001							0.15						
LSD (α = 0.05)	0.76							0.66						

* Means are significantly different from ASR368 F1 or F2 according to Fisher's LSD (α = 0.05)

Table VI.H.8. Flag leaf length (cm) of ASR368 F1 and F2 progeny and three commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Flag leaf length (cm)							Flag leaf length (cm)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	38	5.25	3.00	7.75	1.02	4.92	5.59	40	5.44	2.75	9.00	1.50	4.97	5.92
Backspin	28	6.79*	2.45	9.00	1.40	6.24	7.33	36	5.50	1.50	8.50	1.44	5.01	5.99
Crenshaw	34	6.17*	4.00	11.50	1.62	5.60	6.73	38	5.14	3.00	6.83	1.07	4.79	5.49
Penn A-4	26	5.30	3.00	8.00	1.16	4.83	5.77	36	5.03	2.75	8.50	1.19	4.62	5.43
p value (α = 0.05)	<0.0001							0.33						
LSD (α = 0.05)	0.65							0.60						

* Means are significantly different from ASR368 F1 or F2 according to Fisher's LSD (α = 0.05).

Table VI.H.9. Flag leaf width (mm) of ASR368 F1 and F2 progeny and three commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Flag leaf width (mm)							Flag leaf width (mm)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	38	2.38	1.60	3.70	0.50	2.21	2.55	40	2.48	1.27	4.10	0.60	2.29	2.68
Backspin	28	2.85*	1.23	3.87	0.55	2.64	3.07	36	2.89*	1.60	3.93	0.59	2.69	3.09
Crenshaw	34	2.51	1.45	4.25	0.70	2.26	2.75	38	2.49	1.57	3.97	0.58	2.30	2.68
Penn A-4	26	2.55	1.60	4.00	0.55	2.33	2.77	38	2.84*	1.53	4.55	0.74	2.60	3.08
p value (α = 0.05)		0.01							0.003					
LSD (α = 0.05)		0.29							0.29					

* Means are significantly different from ASR368 F1 or according to Fisher's LSD (α = 0.05).

Table VI.H.10. Flag leaf sheath length (cm) of ASR368 F1 and F2 progeny and three commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Flag Leaf Sheath length (cm)							Flag Leaf Sheath length (cm)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	38	7.94	5.50	9.33	0.86	7.65	8.22	40	8.19	5.83	9.83	1.03	7.86	8.52
Backspin	28	8.35	6.33	10.33	1.10	7.92	8.78	37	7.85	5.00	11.50	1.39	7.39	8.32
Crenshaw	34	8.11	6.33	11.33	1.30	7.66	8.56	38	7.46*	6.17	9.50	0.81	7.19	7.73
Penn A-4	26	8.10	5.83	11.83	1.64	7.44	8.76	38	7.35*	4.67	10.50	1.20	6.96	7.75
p value (α = 0.05)		0.44							0.002					
LSD (α = 0.05)		0.61							0.51					

* Means are significantly different from ASR368 F1 or F2 according to Fisher's LSD (α = 0.05).

Table VI.H.11. Ligule length (mm) of ASR368 F1 and F2 progeny and three commercial creeping bentgrass cultivars in Jefferson County, Oregon in 2002.

Genotype	ASR368 F1 Evaluation							ASR368 F2 Evaluation						
	Ligule Length (mm)							Ligule Length (mm)						
	n	Mean	Min	Max	SD	95% CI of Mean		n	Mean	Min	Max	SD	95% CI of Mean	
ASR368	38	2.13	1.50	2.95	0.38	2.00	2.25	40	2.05	1.30	2.80	0.36	1.94	2.17
Backspin	28	3.16*	2.47	4.33	0.46	2.99	3.34	37	2.61*	1.45	3.57	0.49	2.44	2.77
Crenshaw	34	2.27	1.47	3.23	0.44	2.12	2.42	38	2.02	1.43	2.73	0.35	1.90	2.13
Penn A-4	26	2.17	1.50	3.70	0.45	1.99	2.35	38	1.98	1.17	2.70	0.35	1.86	2.09
p value (α = 0.05)		<0.0001							<0.0001					
LSD (α = 0.05)		0.21							0.18					

* Means are significantly different from ASR368 F1 or F2 according to Fisher's LSD (α = 0.05).

H.3. Experimental methods – greenhouse studies

Plant propagation and establishment

See Section VI.D.1.a for details of the genotypes and plant propagation and establishment methods used in both 2001 and 2002.

Data collected

In 2001 and 2002, data for the number of florets per inflorescence were obtained on three panicles harvested from plants and fixed (Carnoy's solution: 3 volumes 95% ethanol plus 1 volume of glacial acetic acid) for later observation. These observations were made from three batches (repetitions) of plants shipped from Marion County, Oregon to an Iowa greenhouse as described in Section VI.D.1.a.

In 2002, inflorescence length was measured from the base of the basal whorl of panicle branches to the tip of the inflorescence in centimeters. Measurements were made on three fixed panicles from each creeping bentgrass plant.

Statistical analysis

As described in Section VI.D.1.a of this petition.

H.4. Results - greenhouse studies

In 2001, the R0 plants of ASR368 were not significantly different from B99061R and the three commercial cultivars, Penncross, Penn A-4 and Crenshaw, for florets per panicle (TablesVI.H.12 and VI.H.13). The ASR368 F1 RR progeny had significantly more florets per panicle than B99061R and the three commercial cultivars tested. However, mean florets per panicle among ASR368 F1 RR progeny were intermediate to the minimum and maximum number of florets per panicle detected for the commercial cultivars. The difference detected between the ASR368 F1 RR progeny and B99061R was attributed to the genes contributed by the maternal elite parent plants (EPP). In support of this supposition, when maternal elite parent plants were compared directly to the F1 RR progeny in the WA and OR locations described in section VI.F, no significant difference was observed for the number of florets per panicle. In addition, these figures are not out of the ordinary for florets per panicle, since seed set per five panicles indicates means exceeding 450 per panicle for RR and RS phenotypes (Table VI.F.5) The number of florets per panicle presented in table VI.H.14 provides additional evidence that 160 florets per panicle is not atypical of creeping bentgrass.

Consistent with the 2001 results, the number of florets per panicle for the four ASR368 F2 RR progeny lines was not significantly different from the three commercial cultivars in 2002 (Table VI.H.14 and VI.H.15). Several other morphological traits including the

number of anthers and stigmas, the number of glumes, paleas and lemmas were also examined and no unusual characteristics were observed.

Tables VI.H.16 and VI.H.17 provide the mean, standard deviation, minimum and maximum values and specific comparisons between the genotypes tested for panicle length in 2001. Length of the ASR368 R0 was not significantly different from B99061R but was significantly smaller than the commercial cultivars. The ASR368 F1 RR progeny were not significantly different from the control genotypes with regard to panicle length.

Table VI.H.18 contains the mean, standard deviation, minimum and maximum values for inflorescence length taken in 2002. Table VI.H.19 provides the specific comparisons between the genotypes tested for inflorescence length in 2002. The inflorescence length of ASR368 F2 were significantly shorter than those of the commercial cultivars Backspin and Crenshaw but not significantly different from those of Penn A-4.

Table VI.H.12. Mean, standard deviation and the minimum and maximum values for the number of florets per panicle for ASR368 R0 and F1 progeny, B99061R and three commercial creeping bentgrass cultivars in 2001.

Genotype	Number of observations	Mean	Std. deviation	Minimum value	Maximum value
Penn A-4	15	79.78	39.45	29	199
Crenshaw	15	103.75	54.27	44	259
Penncross	14	109.6	45.38	37	197
B99061R	9	99.33	46.18	52	192
ASR368 R0	9	81.84	31.18	46	146
ASR368 F1	9	160.17	60.03	80	250

Table VI.H.13. Comparisons between ASR368 R0 or F1 progeny and B99061R and three commercial creeping bentgrass cultivars for number of florets per panicle in 2001.

Contrast	Mean difference	StdErr	Probt
B99061R vs. ASR368 R0	99.33-81.84 = 17.49	17.61	0.340
B99061R vs. ASR368 F1	99.33-160.17 = -60.84	17.82	0.002*
Penn A-4 vs. ASR368 R0	79.78-81.84 = -2.06	15.68	0.900
Penn A-4 vs. ASR368 F1	79.78-160.17 = -80.39	15.86	<0.0001*
Crenshaw vs. ASR368 R0	103.75-81.84 = 21.91	15.60	0.180
Crenshaw vs. ASR368 F1	103.75-160.17 = -56.42	15.87	0.001*
Penncross vs. ASR368 R0	109.6-81.84 = 27.76	15.89	0.100
Penncross vs. ASR368 F1	109.6-160.17 = -50.57	16.05	0.003*

* Means are significantly different ($\alpha = 0.05$)

Table VI.H.14. Mean, standard deviation, minimum and maximum values for the number of florets per inflorescence between ASR368 F2 RR progeny lines[1] and three commercial creeping bentgrass cultivars in 2002.

Genotype	Number of observations	Mean	Std. deviation	Minimum value	Maximum value
Penn A-4	3	335.67	187.40	160	533
Crenshaw	3	316.00	14.73	300	329
Backspin	3	192.00	41.22	162	239
ASR368 F2 13-2-2	3	309.00	37.40	284	352
ASR368 F2 14-2-6	3	325.33	115.01	211	441
ASR368 F2 15-2-5	3	241.33	25.97	213	264
ASR368 F2 16-2-2	3	189.00	15.39	172	202

[1] 13-2-2, 14-2-6, 15-2-5 and 16-2-2 refer to the maternal EPP of the respective ASR368 F2 progeny.

Table VI.H.15. Estimate difference, standard error and the associated p values of comparisons between ASR368 F2[1] progeny lines and three commercial creeping bentgrass cultivars for the number of florets per panicle in 2002.

Contrast	Mean difference	StdErr	Probt
Penn A-4-vs. ASR368 F2 13-2-2	335.67-309.00 = 26.7	70.77	0.71
Penn A-4 vs. ASR368 F2 14-2-6	335.67-325.33 = 10.3	70.77	0.89
Penn A-4 vs. ASR368 F2 15-2-5	335.67-241.33 = 94.3	70.77	0.20
Penn A-4 vs. ASR368 F2 16-2-2	335.67-189.00 = 146.7	70.77	0.06
Crenshaw vs. ASR368 F2 13-2-2	316.00-309.00 = 7.0	70.77	0.92
Crenshaw vs. ASR368 F2 14-2-6	316.00-325.33 = -9.3	70.77	0.90
Crenshaw vs. ASR368 F2 15-2-5	316.00-241.33 = 74.7	70.77	0.31
Crenshaw vs. ASR368 F2 16-2-2	316.00-189.00 = 127.0	70.77	0.09
Backspin vs. ASR368 F2 13-2-2	192.00-309.00 = -117.0	70.77	0.12
Backspin vs. ASR368 F2 14-2-6	192.00-325.33 = -133.3	70.77	0.08
Backspin vs. ASR368 F2 15-2-5	192.00-241.33 = -49.3	70.77	0.50
Backspin vs. ASR368 F2 16-2-2	192.00-189.00 = 3.00	70.77	0.97

[1] 13-2-2, 14-2-6, 15-2-5 and 16-2-2 refer to the maternal EPP of the respective ASR368 F2 progeny.

Table VI.H.16. Number of observations, mean, standard deviation and the minimum and maximum values for inflorescence length for ASR368 R0 and F1 progeny, B99061R and three commercial creeping bentgrass cultivars in 2001.

Genotype	Number of observations	Mean (cm)	Std deviation	Minimum value	Maximum value
Penn A-4	15	5.16	1.22	2.9	8.0
Crenshaw	15	5.24	1.26	3.4	7.5
Penncross	14	5.32	1.15	3.0	7.7
B99061R	9	4.98	1.08	3.6	6.5
ASR368 R0	9	4.23	0.62	3.0	5.0
ASR368 F1	9	5.36	0.92	3.9	6.6

Table VI.H.17. Mean difference, standard error and the p value associated with each comparison between ASR368 R0 or F1 progeny and B99061R and three commercial creeping bentgrass cultivars for inflorescence length in 2001.

Contrast	Mean difference	StdErr	Probt
B99061R vs. ASR368 R0	4.98-4.23 = 0.75	0.51	0.16
B99061R vs. ASR368 F1	4.98-5.36 = -0.38	0.47	0.42
Penn A-4 vs. ASR368 R0	5.16-4.23 = 0.93	0.42	0.04*
Penn A-4 vs. ASR368 F1	5.16-5.36 = -0.20	0.37	0.59
Crenshaw vs. ASR368 R0	5.24-4.23 = 1.01	0.42	0.02*
Crenshaw vs. ASR368 F1	5.24-5.36 = -0.12	0.37	0.76
Penncross vs. ASR368 R0	5.32-4.23 = 1.09	0.42	0.02*
Penncross vs. ASR368 F1	5.32-5.36 = -0.04	0.37	0.93

* Means are significantly different ($\alpha = 0.05$)

Table VI.H.18. Mean, standard deviation, minimum and maximum values for inflorescence length between ASR368 F2 progeny lines[1] and three commercial creeping bentgrass cultivars in 2002.

Genotype	Number of observations	Mean (cm)	Std deviation	Minimum value	Maximum value
Penn A-4	3	7.83	2.00	5.9	9.9
Crenshaw	3	7.90	0.85	7.1	8.8
Backspin	3	8.07	0.78	7.2	8.7
ASR368 F2 13-2-2	3	6.70	0.36	6.4	7.1
ASR368 F2 14-2-6	3	6.10	0.75	5.4	6.9
ASR368 F2 15-2-5	3	6.40	1.35	4.9	7.5
ASR368 F2 16-2-2	3	6.80	0.78	5.9	7.3

[1] 13-2-2, 14-2-6, 15-2-5 and 16-2-2 refer to the maternal EPP of the respective ASR368 F2 progeny.

Table VI.H.19. Mean difference, standard error and the associated p values of comparisons between four ASR368 F2 progeny lines[1] and three commercial creeping bentgrass cultivars for inflorescence length in 2002.

Contrast	Mean difference	StdErr	Probt
Penn A-4- vs. ASR368 F2 13-2-2	7.83-6.70 = 1.13	0.90	0.23
Penn A-4 vs. ASR368 F2 14-2-6	7.83-6.10 = 1.73	0.90	0.07
Penn A-4 vs. ASR368 F2 15-2-5	7.83-6.40 = 1.43	0.90	0.13
Penn A-4 vs. ASR368 F2 16-2-2	7.83-6.80 = 1.03	0.90	0.27
Crenshaw vs. ASR368 F2 13-2-2	7.90-6.70 = 1.20	0.90	0.20
Crenshaw vs. ASR368 F2 14-2-6	7.90-6.10 = 1.80	0.90	0.06
Crenshaw vs. ASR368 F2 15-2-5	7.90-6.40 = 1.50	0.90	0.12
Crenshaw vs. ASR368 F2 16-2-2	7.90-6.80 = 1.10	0.90	0.24
Backspin vs. ASR368 F2 13-2-2	8.07-6.70 = 1.37	0.90	0.15
Backspin vs. ASR368 F2 14-2-6	8.07-6.10 = 1.97	0.90	0.05*
Backspin vs. ASR368 F2 15-2-5	8.07-6.40 = 1.67	0.90	0.08
Backspin vs. ASR368 F2 16-2-2	8.07-6.80 = 1.27	0.90	0.18

[1] 13-2-2, 14-2-6, 15-2-5 and 16-2-2 refer to the maternal EPP of the respective ASR368 F2 progeny.

* Means are significantly different ($\alpha = 0.05$)

H.5. Overall conclusion for botanical characteristics

In field trials at two locations, ASR368 F1 and F2 RR progeny were significantly different from populations of commercial creeping bentgrass cultivars on few occasions for each of the parameters measured or observed within the Washington and Oregon locations other than for ligule length. However, the ligule length observed for all of the plants in the study was typical of *A. stolonifera,* according to Hitchcock (1950) and not likely to affect the weed or plant pest potential of ASR368. Visual inspection and evaluation of typical botanical features expected for creeping bentgrass indicate no gross aberration or deviation in any plant morphological feature between the ASR368 genotypes and commercial cultivars. In the Iowa State University greenhouse study, differences were not detected in the number of florets per panicle between ASR368 R0, B99061R and the commercial cultivars.

Therefore, except for imparting the Roundup Ready trait to ASR368 and its progeny, the insertion and expression of the *cp4 epsps* gene did not alter the morphology, floral or vegetative features in an appreciable way. The results of these studies further demonstrate that the measured botanical characteristics of ASR368 and its progeny are within the normal range of the commercial creeping bentgrass cultivars or other non-transgenic creeping bentgrasses and should not be expected to be different from them. Finally, these results support a conclusion of no contribution to increased weed potential

based on these botanical characteristics of ASR368 compared to commercial creeping bentgrass cultivars that are representative of *A. stolonifera.*

I. Disease and Pest Susceptibility of ASR368

Observations of plant growth and insect and disease susceptibility were made during field releases conducted between 1999 through 2002. Data collected from these trials demonstrated that plants of ASR368 grew normally and exhibited the expected morphology, phenotype, and physical characteristics of conventional creeping bentgrass. Susceptibility to diseases and insects remained unchanged compared to non-transgenic creeping bentgrasses. Observations on plant growth characteristics and weediness potential provided evidence that ASR368 does not pose a plant pest risk, or exhibit enhanced weediness characteristics.

The first field release of ASR368 occurred in the United States during 1999. The notification number and release site information for ASR368 can be found in Table VI.I.1. Field data reports have been submitted to USDA/APHIS for field trials conducted in 1999 through 2002 as required. Final reports have not yet been submitted for ongoing field trials for the 2002-2003 seasons. These reports will be submitted within the designated time frame.

In all field releases of ASR368 conducted under notification, researchers were requested to monitor field sites for pest susceptibility, aberrant plant characteristics, or changes in the weediness characteristic of ASR368. Field observations were also made on the incidence of beneficial organisms. Visual observations were made while walking the fields and, in almost all circumstances; the observations were qualitative rather than quantitative.

Plant health management in professionally managed turf is distinct from that of row crops, trees, nuts, and vines. Golf course superintendents are trained and expert in the identification of potential problems in golf course turfgrass. They consider injury or damage caused by biotic agents as unacceptable, while in traditional agriculture the need for intervention is a decision driven more by economic injury levels. When considering that every golf superintendent desires a uniform and consistent playing surface of creeping bentgrass, the list of potential plant pests is relatively long.

The major turf diseases of consistent concern affecting creeping bentgrass performance are dollar spot (*Sclerotinia homocarpa*), brown patch (*Rhizoctonia solani*), snow mold (*Myriosclerotinia borealis, Typhula incarnata)*, leafspots (*Helminthosporium sp.*, *Dreschelera sp., Septoria sp.*), take-all patch (*Gaeumannomyces graminis*), copper spot (*Gloeocercospora sorghil*), leaf rust (*Puccinia sp.*), bentgrass dead spot (*Leptosphaeria narmari*), and pythium (*Pythium sp*).

Insect pests of major importance affecting the performance of creeping bentgrass are chinch bugs (*Blissus leucopterus*), various beetle grubs (*Popillia spp.*), sod webworms

(*Crambus spp.*), cutworms (*Agriotis ipsilon and Peridroma saucia*), armyworms (*Spodoptera spp.*), billbugs (*Spheophorus spp.*), mole crickets (*Scapteristicus spp.*), and aphids (*Aphidius spp.*).

The diseases and insects observed during these field trials are summarized in Table VI.I.1. No discernible differences in plant growth, disease severity or insect infestation were detected between ASR368 and non-transgenic control plants in these trials. Although this information is qualitative, many of the research cooperators conducting these experiments were turf scientists or turfgrass managers experienced in the evaluation of new turfgrass varieties and performance of bentgrasses. Many of them have participated in the National Turfgrass Evaluation Program trials.

Finally, based on the information gathered over three years of monitoring ASR368 field trials, we would expect ASR368 varieties to exhibit the morphology, phenotype, and physical characteristics of commercial creeping bentgrass cultivars.

Table VI.I.1. Differences in disease and insect susceptibility and plant growth characteristics observed between ASR368 and non-transgenic bentgrasses.

USDA Notification #	Year	State	County	Disease[1] Δ	Insect[2] Δ	Growth[3] Δ
99-203-04n	1999-2000	NJ	Middlesex	no	no	no
99-203-04n	1999-2000	OH	Union	no	no	no
99-203-04n	1999-2000	OR	Marion	no	no	no
00-159-02n	2000-2001	IL	Clinton	no	no	no
00-159-02n	2000-2001	MI	Ottawa	no	no	no
00-201-03n	2000-2001	NJ	Middlesex	no	no	no
00-201-03n	2000-2001	OH	Union	no	no	no
00-201-03n	2000-2001	OR	Marion	no	no	no
00-220-02n	2000-2001	OR	Jefferson	no	no	no
00-220-02n	2000-2001	WA	Franklin	no	no	no
00-224-01N	2000-2001	AL	Baldwin	no	no	no
00-224-01n	2000-2001	CO	Larimer	no	no	no
00-224-01n	2000-2001	IN	Tippecanoe	no	no	no
00-224-01n	2000-2001	KY	Fayette	no	no	no
00-224-01n	2000-2001	MA	Franklin	no	no	no
00-224-01n	2000-2001	MD	Prince George	no	no	no
00-224-01n	2000-2001	MI	Ingham	no	no	no
00-224-01n	2000-2001	NJ	Middlesex	no	no	no
00-224-01n	2000-2001	NY	Tompkins	no	no	no
00-224-01n	2000-2001	OH	Franklin	no	no	No
00-224-01n	2000-2001	OR	Linn	no	no	No
00-224-01n	2000-2001	OR	Marion	no	no	no

[1] Diseases observed for included: dollar spot, brown patch, snow mold, leaf spot, take-all patch, copper spot, rust, spring dead spot, Pythium.
[2] Δ = difference observed between transgenic and control plants
[3] Insects observed for included: chinchbugs, grubs, sod webworms, cutworms, armyworms, billbugs, mole crickets, aphids, lady beetles, spiders, honeybees.
[4] Plant growth characteristics observed for turf included: rate of germination and establishment and flowering when mowed and plant growth. Characteristics observed for seed production included: rate of establishment, spread, flowering and yield.

Table VI.I.1. (contd.) Differences in disease and insect susceptibility and plant growth characteristics observed between ASR368 and non-transgenic bentgrasses

USDA Notification #	Year	State	County	Disease[1] Δ	Insect[2] Δ	Growth[3] Δ
00-224-01n	2000-2001	OR	Umatilla	no	no	no
00-224-01n	2000-2001	VA	Montgomery	no	no	no
00-224-01n	2000-2001	WI	Dane	no	no	no
00-272-05n	2000-2001	NE	Saunders	no	no	No
01-064-02n	2001-2002	IL	Champaign	no	no	no
01-064-02n	2001-2002	NC	Wake	no	no	no
01-151-02n	2001-2002	IL	Clinton	no	no	no
01-151-02n	2001-2002	MI	Ottawa	no	no	no
01-177-01n	2001-2002	OR	Jefferson	no	no	no
01-177-01n	2001-2002	WA	Franklin	no	no	no
01-177-02n	2001-2002	AL	Baldwin	no	no	no
01-177-02n	2001-2002	FL	Orange	no	no	no
01-177-02n	2001-2002	IA	Story	no	no	no
01-177-02n	2001-2002	IL	Champaign	no	no	no
01-177-02n	2001-2002	IN	Tippecanoe	no	no	no
01-177-02n	2001-2002	KY	Fayette	no	no	no
01-177-02n	2001-2002	MD	Prince George	no	no	No
01-177-02n	2001-2002	MI	Ingham	no	no	no
01-177-02n	2001-2002	NC	Wake	no	no	no
01-177-02n	2001-2002	NE	Saunders	no	no	no
01-177-02n	2001-2002	NJ	Middlesex	no	no	no

[1] Diseases observed for included: dollar spot, brown patch, snow mold, leaf spot, take-all patch, copper spot, rust, spring dead spot, Pythium.
[2] Δ = difference observed between transgenic and control plants
[3] Insects observed for included: chinchbugs, grubs, sod webworms, cutworms, armyworms, billbugs, mole crickets, aphids, lady beetles, spiders, honeybees.
[4] Plant growth characteristics observed for turf included: rate of germination and establishment and flowering when mowed and plant growth. Characteristics observed for seed production included: rate of establishment, spread, flowering and yield.

Table VI.I.1. (contd.) Differences in disease and insect susceptibility and plant growth characteristics observed between ASR368 and non-transgenic bentgrasses.

USDA Notification #	Year	State	County	Disease Δ	Insect Δ	Growth Δ
01-177-02n	2001-2002	NY	Tompkins	no	no	no
01-177-02n	2001-2002	OH	Franklin	no	no	no
01-177-02n	2001-2002	OH	Union	no	no	no
01-177-02n	2001-2002	OR	Marion	no	no	no
01-177-02n	2001-2002	OR	Linn	no	no	no
01-177-02n	2001-2002	OR	Umatilla	no	no	no
01-177-02n	2001-2002	SC	Pickens	no	no	No
01-177-02n	2001-2002	VA	Montgomery	no	no	no
01-177-02n	2001-2002	WA	Franklin	no	no	no
01-177-02n	2001-2002	WI	Dane	no	no	no
01-199-06n	2001-2002	IA	Polk	no	no	no
01-199-06n	2001-2002	IL	Cook	no	no	no
01-199-06n	2001-2002	KY	Boone	no	no	no
01-199-06n	2001-2002	MD	Baltimore	no	no	no
01-199-06n	2001-2002	NJ	Union	no	no	no
01-199-06n	2001-2002	NY	Broome	no	no	no
01-199-06n	2001-2002	OH	Delaware	no	no	no
01-199-06n	2001-2002	OH	Fairfield	no	no	no
01-199-06n	2001-2002	OH	Union	no	no	no
01-199-06n	2001-2002	WI	Sheboygen	no	no	no
01-214-01n	2001-2002	DE	New Castle	no	no	no
01-214-01n	2001-2002	MD	Montgomery	no	no	no

[1] Diseases observed for included: dollar spot, brown patch, snow mold, leaf spot, take-all patch, copper spot, rust, spring dead spot, Pythium.
[2] Δ = difference observed between transgenic and control plants
[3] Insects observed for included: chinchbugs, grubs, sod webworms, cutworms, armyworms, billbugs, mole crickets, aphids, lady beetles, spiders, honeybees.
[4] Plant growth characteristics observed for turf included: rate of germination and establishment and flowering when mowed and plant growth. Characteristics observed for seed production included: rate of establishment, spread, flowering and yield.

J. Section VI Conclusion

The experiments and observations described in Chapters A through I of Section VI were performed to help establish greater familiarity with Roundup Ready creeping bentgrass event ASR368 and to better understand its plant pest or weed potential in comparison to non-transgenic creeping bentgrasses representing the range in variability common to *A. stolonifera.* All stages of the creeping bentgrass life cycle that could enhance the weed or plant pest potential of ASR368 compared to non-transgenic creeping bentgrasses were examined. Comparisons were made with regard to: seed and vegetative establishment, plant growth, flowering, pollen viability and longevity, fecundity, seed germination and seedling vigor, botanical characteristics, insect resistance, and disease susceptibility. University scientists in addition to those of Monsanto and The Scotts Company performed more than ninety individual experiments between 1999 and 2003 at 65 field locations representing the northern or cool, southern or warm and transition zone climates of turfgrass adaptation (Beard, 1982). Finally, the environments these experiments were performed in consisted of both managed and unmanaged ecosystems with variations of light, moisture, soils, nutrition, competition and temperature extremes.

The results of these studies provide a solid basis for determining that the weed or plant pest potential of event ASR368 and its progeny is not different from that of non-transgenic creeping bentgrasses representative of *A. stolonifera*. The following paragraphs briefly summarize the findings reported in the nine Chapters of Section VI.

The results of bare soil and competitive turf seed establishment studies conducted in 2001 and 2002 at four total locations encompassing irrigated and non-irrigated conditions, variation in competition and seasonal establishment were presented in Chapter VI.A. These studies demonstrated that: (1) the establishment and persistence of ASR368 tended to fall within the range of the commercial cultivars, (2) the establishment and persistence of ASR368 was generally low for all genotypes when seeded in bare soil (< 30%) and (3) the establishment and persistence of seedlings of all genotypes was completely unsuccessful when seeded into an existing competitive situation. Given the results from these experiments, which further confirm reports in the scientific literature cited in Chapter VI.A, seed of ASR368 would not be expected to germinate, establish or persist in unmanaged competitive and non-competitive ecosystems differently from non-transgenic creeping bentgrasses. These findings support a conclusion of no contribution to increased weed potential of ASR368 and its progeny compared to commercial creeping bentgrass cultivars representative of *A. stolonifera* based on these seed establishment characteristics.

The results of several vegetative establishment studies conducted in 2001 and 2002 at six different locations including irrigated and non-irrigated conditions were presented in Chapter VI.B. These studies demonstrated that: (1) the vegetative establishment and persistence of ASR368 tended to fall within the range of the non-transgenic genotypes, (2) ASR368 plants are not different from commercial or non-transgenic creeping bentgrass cultivars in their ability to produce new tillers from viable stolon nodes and (3) the establishment of all creeping bentgrass genotypes was much reduced under non-

irrigated versus irrigated conditions. Given the results from these experiments, which further confirm reports in the scientific literature cited in Chapter VI.B, ASR368 would not be expected to vegetatively establish or persist differently from non-transgenic creeping bentgrasses. These findings further support a conclusion of no contribution to increased weed potential of ASR368 and its progeny compared to commercial creeping bentgrass cultivars representative of *A. stolonifera* based on its ability to vegetatively establish.

The growth of ASR368 was compared to non-transgenic bentgrasses in bare soil and competitive turf at eleven locations representing the northern (cool), southern (warm) and transition climate zones in 2000, 2001, 2002 and 2003 in Chapter VI.C. These studies demonstrated that: (1) ASR368 displayed no increase in vegetative growth, aggressiveness, invasiveness or relative fitness compared to commercial creeping bentgrass cultivars when established in bare soil with no competition or with competition from other turfgrasses in cool, warm or transition climates, (2) ASR368 displayed no competitive advantage in comparison to commercial creeping bentgrass cultivars in direct sun vs. shade or reduced light and (3) the relative growth of ASR368 is within the normal range for other commercially available creeping bentgrass cultivars. Given the results of these experiments, which further confirm reports in the scientific literature cited in Chapter VI.C, ASR368 and its progeny would not be expected to grow in a different manner in either managed or unmanaged ecosystems from commercial creeping bentgrass cultivars. Consequently, these findings support a conclusion of no contribution to increased weed potential of ASR368 and its progeny based on their growth relative to commercial creeping bentgrass cultivars representative of *A. stolonifera*.

The flowering characteristics of ASR368 and non-transgenic creeping bentgrass genotypes evaluated in the greenhouse and at four different field locations in 2001 and 2002 were reported in Chapter VI.D. These studies demonstrated that: (1) ASR368 genotypes were within the range of B99061R and the commercial cultivars for date of first inflorescence, anthesis initiation, anthesis completion and the duration of anthesis; (2) were consistent with the findings of Christoffer (2003) who observed that the flowering characteristics of ASR368 and a number of other *Agrostis* species were not different in Washington in 2001 and 2002; and (3) ASR368 and its progeny should not be expected to flower differently from non-transgenic creeping bentgrasses. Given the results from these experiments, which further confirm reports in the scientific literature cited in Chapter VI.D, ASR368 would not be expected to flower differently from non-transgenic creeping bentgrasses. Consequently, these data and information support a conclusion of no contribution to increased weed potential of ASR368 and its progeny compared to commercial creeping bentgrass cultivars representative of *A. stolonifera* based on its flowering characteristics.

The size and longevity of pollen collected from plants of ASR368 and non-transgenic creeping bentgrass genotypes, grown in the greenhouse and field, evaluated in 2001 and 2002 were reported in Chapter VI.E. These studies demonstrated that: (1) the diameter of pollen from ASR368, B99061R and commercial creeping bentgrass cultivars was not significantly different; (2) the longevity of pollen from ASR368, B99061R and

commercial creeping bentgrass cultivars was not significantly different and (3) the size and longevity of pollen from ASR368 or its progeny are within the normal ranges of these characteristics compared to commercial creeping bentgrass cultivars and other non-transgenic creeping bentgrasses.

Given the results from the ASR368 pollen experiments, which are consistent with reports in the scientific literature cited in Chapter VI.E regarding the longevity of grass pollen, ASR368 pollen would not be expected to be larger, smaller, or viable for a longer period of time than pollen from non-transgenic creeping bentgrasses. These findings support a conclusion of no contribution to increased weed or dispersal potential of ASR368 and its progeny compared to commercial creeping bentgrass cultivars representative of *A. stolonifera* based on the size and longevity of its pollen.

Results of the fecundity characteristics of ASR368 evaluated in the greenhouse and at four different field locations in 2001 and 2002 were reported in Chapter VI.F. These studies demonstrated that: (1) ASR368 open-and self- pollinated seed set is not significantly different from B99061R or the commercial cultivars; (2) ASR368 self-pollinated seed set is low, as expected, due to self-incompatibility systems known to exist in the *Agrostis* genus and (3) seed production is variable among creeping bentgrass cultivars and that the ASR368 genotypes are within the range of the commercial cultivars evaluated that are representative of *A. stolonifera*. Given the results of the 2001 and 2002 fecundity studies, which encompassed three generations of ASR368, it is not expected that ASR368 and its progeny would differ in their ability to produce seed compared to commercial cultivars. This supports a conclusion of no contribution to increased weed potential based on the seed characteristics of ASR368 compared to commercial creeping bentgrass cultivars representative of *A. stolonifera*.

Physiological characteristics of the seed of ASR368 were compared to non-transgenic genotypes in a number of germination tests performed in the laboratory. The results of these evaluations were reported in Chapter VI.G. These studies demonstrated that: (1) ASR368 RR seed does not germinate differently than non-transgenic ASR368 RS seed under, standard, suboptimal and supra-optimal conditions; (2) the survival and germination of ASR368 RR seed were not different than those of ASR368 RS seed under the stressful conditions of the Accelerated Aging Test, which is also a measure of seed longevity and (3) the germination energy and seedling vigor of ASR368 RR seed were not significantly different across germination conditions from those of the ASR368 RS. Given the results of the seed physiology studies, it is not expected that seed of ASR368 would differ in their ability to germinate under stressful conditions or have greater longevity than seed of non-transgenic creeping bentgrasses. This supports a conclusion of no contribution to increased weed potential based on the seed characteristics of ASR368 compared to non-transgenic creeping bentgrass cultivars representative of *A. stolonifera.*

The results of the ASR368 botanical characteristic evaluations conducted in the greenhouse and at four different field locations in 2001 and 2002 are presented in Chapter VI.H. These studies demonstrated that: (1) the visual inspection and evaluation of a

number of botanical characteristics indicate no gross aberration or deviation between the ASR368 genotypes and commercial cultivars and (2) insertion and expression of the cp4 epsps gene did not alter the morphology, floral or vegetative features of ASR368 in a significant way. Given the results from these experiments, which further confirm reports in the scientific literature cited in Chapter VI.H, the botanical characteristics of ASR368 would not be expected to be different from non-transgenic creeping bentgrasses. This supports a conclusion of no contribution to increased weed potential based on the botanical characteristics of ASR368 compared to non-transgenic creeping bentgrass cultivars representative of *A. stolonifera*.

Finally, in Chapter VI.I, the observations of plant growth, and insect and disease susceptibility conducted for 65 field releases performed between 1999 and 2002 are summarized. These observations demonstrate that: (1) there are no discernible differences in plant growth between ASR368 and non-transgenic plants; (2) there are no discernible differences in disease severity between ASR368 and non-transgenic plants and (3) there are no discernible differences in insect infestation between ASR368 and non-transgenic plants. These results contribute to a conclusion that ASR368 and its progeny would be expected to exhibit the morphology, phenotype, and physical characteristics of commercial or non-transgenic creeping bentgrass cultivars representative of *A. stolonifera*.

Based on the results and observations from this comprehensive series of studies and reports in the scientific literature, it can be concluded that event ASR368 and its progeny are not different from commercial or non-transgenic creeping bentgrasses with regard to the characteristics evaluated and presented in the chapters of Section VI, other than tolerance to glyphosate herbicide. It can be further concluded that event ASR368 and its progeny exhibit no plant pathogenic properties, are no more likely to become a weed than non-transgenic creeping bentgrasses, are unlikely to increase the weediness of any other cultivated plant or native wild species with which *A. stolonifera* can interbreed and are unlikely to harm other organisms that are beneficial to agriculture.

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VII. Environmental Consequences of Introduction of ASR368

A. Adoption of Roundup Ready crops producing the CP4 EPSPS protein

Growers have rapidly adopted Roundup Ready crops due to the simplicity they offer in weed control. Roundup agricultural and industrial, turf and ornamental herbicides are highly effective against the majority of annual and perennial weeds. The actual benefits that Roundup Ready creeping bentgrass seed producers and golf courses realize cannot be measured until after the product has been commercialized. However, Runge and Fawcett (1998) and Gianessi and Carpenter (2000) have documented many of the benefits realized by the adoption of Roundup Ready soybean (*Glycine max*) that can be related to creeping bentgrass. These benefits may include enhanced weed control, reduced soil erosion, improved soil quality, improved water quality, increased seed yield, improved farm efficiency and/or reduced farm production costs.

Growers throughout the world have realized substantial benefits with other Roundup Ready crops as evidenced by their broad adoption. Starting with the commercial introduction of Roundup Ready soybean in 1996 and followed by Roundup Ready canola, corn and cotton, these crops have been planted on more than 400 million acres worldwide (Monsanto unpublished data, 2003).

B. Weed potential of ASR368

Since the introduction of Roundup Ready crops, there have been no reports of these crops being weedier than their conventional counterparts or that they have altered the invasiveness of any species with which they can interbreed. Section II of this petition discusses the characteristics of weed and invasive species and specifically the weediness potential of creeping bentgrass. In this section, we discuss whether the introduction and expression of the *cp4 epsps* gene and production of the encoded CP4 EPSPS protein could cause Roundup Ready creeping bentgrass event ASR368 to differ in weediness to conventional creeping bentgrass. We will also discuss the implications of trait transfer to hybrids that may form with *A. stolonifera*.

Approximately 95% of the creeping bentgrass seed produced in the U.S. each year is grown in the Willamette Valley of Oregon. This area has been the predominant creeping bentgrass seed production location for the past 75 years (Schoth, 1930). Throughout this period, there has been no demonstration that random hybridization within *A. stolonifera* or related species has either increased aggressiveness or been detrimental to other species in that environment. Furthermore, within the continental U.S., *A. stolonifera* is not listed as a serious, principle or common weed (Holm *et al.*, 1979). Furthermore, the manual, Weeds of the Northeast (Uva et al, 1997), does not discuss creeping bentgrass (or any *Agrostis* species) except to distinguish it from another species.

In more than 90 experiments conducted since 1999, there has been no indication that introduction of the *cp4 epsps* gene into creeping bentgrass altered the weed potential of the plant (Section VI). In addition, compared to non-transformed plants or commercial bentgrass cultivars, no fitness advantage has been noted with creeping bentgrass event ASR368 with respect to: (1) establishment via seed or stolons, (2) relative growth and persistence, (3) flowering (initiation, beginning anthesis and duration), (4) pollen biology (size and viability/longevity), (5) seed set (duration and yield), (6) seed physiology (longevity, vigor and dormancy) and (7) botanical characteristics. Therefore, other than its tolerance to glyphosate herbicide, event ASR368 imparts no known advantages in any of these categories that might contribute to increased ecological fitness or weedy or invasive potential across a range of environments in the absence of the herbicide selective pressure. Furthermore, plants do not encounter glyphosate in unmanaged ecosystems, thus there should be no natural mechanism to select for tolerant individuals over susceptible ones.

C. Potential of gene transfer from ASR368 to wild and cultivated related species

C.1. Introduction

As discussed in Section II, creeping bentgrass is a highly self-incompatible, essentially obligate outcrossing and wind-pollinated species. Consequently, within the United States the potential for outcrossing among *Agrostis spp.* is recognized and low levels of gene flow have been viewed as an acceptable risk in seed production (Knowles, 1966). Studies by Wipff and Fricker (2000) and Belanger *et al.* 2003) demonstrate that a low level of creeping bentgrass outcrossing to creeping bentgrass (*Agrostis stolonifera* to *A. stolonifera*) occurs between cultivars at distance. Wipff and Fricker (2000) identified a single intraspecific hybrid plant among more than a thousand at a distance of 958 feet from the pollen source.

There have been reports of inter-specific crossing within the genus *Agrostis* by a number of authors (Davies, 1953; Jones 1956 a, b, c; Bradshaw, 1958a; Tutin, 1980; Wipff and Fricker, 2000; Belanger *et al.* 2003). In the studies conducted by Davies (1953), Jones (1956a,b, c), Bradshaw (1958a) and Tutin (1980), these hybrids were artificially produced and were typically of intermediate morphology between the two parents. In addition, they either had a complete loss or much reduced fertility. Inter-generic hybrids have also been reported between *A. stolonifera* and *Polypogon spp.* but the hybrids were sterile (Björkman, 1960).

C.2. Event ASR368 transgene flow to *A. stolonifera* and related species

To evaluate the potential for pollen-mediated transgene flow from event ASR368 to other related species, a two-year study was initiated with researchers at Washington State University in Franklin County, WA during 2000 – 2002 (USDA# 00-224-01n and 01-177-02n). The goals of this study were to examine: (1) the potential for pollen-mediated event ASR368 intraspecific, interspecific and intergeneric transgene flow and (2) the effect of distance from the "pollen (transgene) donor" on hybridization potential

compared with conventional *Agrostis spp.* and *Polypogon spp*. A summary of the results of this study is provided below and was excerpted from the Masters of Science thesis by Chistoffer (2003).

C.2.a. Experimental methods

To evaluate the potential for RRCB pollen-mediated transgene flow, a 50-ha irrigated field was planted in late August 2000 with 'Chateau' Kentucky bluegrass (*Poa pratensis* L.) in Franklin County, WA. In early October 2000, glyphosate at 841 g ae ha^{-1} was sprayed directly onto the Kentucky bluegrass where the RRCB pollen source and the 15 species used as pollen receptors were to be planted. All plots were later tilled to loosen the soil for planting.

On October 24, 2000, a central planting (13.7-m radius) of approximately 400, two-month old seedlings of F1 RRCB progeny hemizygous for the *cp4 epsps* gene were planted as a pollen (transgene) donor. Seedlings of 12 different *Agrostis spp*. and three *Polypogon spp*. served as "pollen (transgene) receptor" plants. These species were planted as a five-plant row within 24 x 14 m plots (78.7 x 46 feet) at 50, 274 and 354 m (164, 899 and 1161 feet) from the edge of the RRCB pollen donor plants along six axes radiating from the field center at 60-degree intervals. A single plot was also established in the direction of the prevailing wind 0.5 m from the RRCB on the 0 line (0-line 2 m plot). All plots were approximately 23 x 14 m (Figure VII.C.1.a).

On March 21, 2001, separate five-plant rows of bentgrass cultivars Crenshaw and SR1020 were planted into new 8- x 8-m transgene receptor plots 185 m from the transgene donor RRCB at all axes to supplement the study. Between the Crenshaw and SR1020 rows, 'Southshore', 'Pennlinks', 'Putter', and 'Providence' *A. stolonifera* were added as single five-plant rows. On April 13, 2001 additional receptor and RRCB plants were added to supplement the study, or as replacements for winter-killed plants. The additional RRCB plants were placed 0.5 m inside the first RRCB circle. For the same reasons, additional plants were transplanted in many of the plots during March to May 2002 (Figure VII.C.1.b).

Plants were harvested, from late June until early August of 2001 and 2002, when visual inspection demonstrated that greater than 65% of the panicles were mature. Panicles were cut near the base, placed into a paper bag, and each five-plant row within a plot was placed into a separate cloth bag. Seed from each plant were planted in the greenhouse, seedlings counted and then sprayed with glyphosate, nine days after emergence and again at 28 days after planting. The percentage of surviving seedlings was used to determine intraspecific, interspecific, and intergeneric transgene flow from event ASR368. Surviving seedlings were also transplanted to evaluate the vegetative and plant growth characteristics of these hybrids in comparison to the parental species. The results of this evaluation are presented in the following section and in Appendix IV.

C.2.b. Results

In both 2001 and 2002, the percentage of intraspecific RR progeny recovered within the *A. stolonifera* pollen receptor plants was greatest on the 0 and 60-degree lines (Table VII.C.1.a and b). The percentage of RR progeny recovered decreased with greater distance from the ASR368 pollen source. In 2001, intraspecific RR progeny recovery was 48.9% at one to three meters and decreased to 0.07% at 354 meters on the single line aligned with the prevailing wind (Table VII.C.1.a). In 2002, intraspecific RR progeny recovery was 26.6% at 1 to 3 meters, 0.94% and 2.74% at 50 meters and 0.01% and 0.15% at 354 meters on the 0 and 60-degree lines, respectively (Table VII.C.1.b). The percentage of intraspecific RR progeny formation is consistent between years and with the findings of Belanger *et al.* (2003) described in Section II.E.6 of this petition.

In both 2001 and 2002, interspecific and intergeneric transgene flow were considerably less than intra-specific transgene flow and both also decreased significantly with distance from the pollen source plants. In 2001, event ASR368 formed interspecific and intergeneric hybrids with *A. trinii*, *A. pallida*, *A. idahoensis*, *A. pallens*, *A. capillaris, A. gigantea*, *A. canina*, *A. hyemalis var. scabra, A. vinealis*, *Polypogon monspeliensis*, *P. fugax,* and *P. viridis* at one to three meters from the event ASR368 pollen source (Table VII.C.2). No RR hybrids were formed with *A. castellana* or *A. nebulosa* in 2001. At 50 meters, RR hybrids were recovered from *A. capillaris*, *A. hyemalis var. scabra*, *A. idahoensis*, *P. fugax,* and *P. viridis* at less than 0.05% and only at the 0-line 50-m plot (Table VII.C.3). No interspecific or intergeneric progeny were recovered beyond 50 m.

In 2002, at 1 to 3 meters, event ASR368 formed interspecific and intergeneric hybrids with *A. idahoensis*, *A. pallens*, *A. capillaris*, *A. gigantea*, *A. canina*, and, *P. monspeliensis*, *P. fugax* and *P. viridis* (Table VII.C.2). No RR hybrids were formed with *A. trinii, A. castellana* and *Apera interrupta* at this distance. At 50 meters on the 0-line, RR hybrids were recovered from *P. fugax, P. monspeliensis, P. viridis, A. capillaris,* and *A. gigantea* (Table VII.C.3). RR progeny were also recovered from *A. idahoensis* at the 60- and 300-lines 50-m plot at 0.004 and 0.003%, respectively. No interspecific or intergeneric progeny were recovered beyond 50 m.

C.2.c. Conclusions from hybridization studies

These data suggest that the potential for interspecific and intergeneric hybridization beyond 50 m is low and much less likely to occur than intraspecific hybridization. These rates of interspecific or intergeneric hybridization are also consistent with the findings of Belanger *et al.* (2003; described in Section II.E.6 of this petition).

It is interesting to note that the current OSCS Handbook (2001) guidelines for *A. stolonifera* seed production fields requires the same isolation distance between all *Agrostis* spp. seed production fields. No distinction is made between intraspecific and interspecific gene flow potential. These data and those of Belanger *et al.* (2003) suggest that the isolation distance between *A. stolonifera* and other *Agrostis* spp. seed production field is excessively stringent.

C.3. Characteristics of hybrids recovered from event ASR368 and related species

Comparative evaluation of Roundup Ready event ASR368 to commercial creeping bentgrass cultivars representative of *A. stolonifera* revealed no selective advantage conferred by the introduced *cp4 epsps* gene and expression of the CP4 EPSPS protein. Thus, it would be expected that no advantage would be conferred to a hybrid formed between ASR368 and a related species with which it can interbreed. However, APHIS requested confirmatory data on hybrid development that is presented here.

The glyphosate tolerant hybrid seedlings from several representative plants of each species and the parent species were transplanted to individual plastic pots. Three replications, each containing 20 plants of each seedling lot were arranged in a randomized complete block design. The plants were allowed to develop in a poly-house in Marion County, Oregon prior to estimation of ground coverage, growth habit and tiller density. The conventional (non-transgenic) parent species used as reference comparisons for the recovered transgenic hybrids are provided in Table VII.C.4.

Data were collected for the following parameters: (1) percent ground cover, (2) tillers per plant and (3) plant growth habit. Vegetative spring growth was estimated as percent cover of each container by the plant represented by the parental species collections or the recovered hybrids. Tiller capacity per plant was estimated using a scale of 1-5 where, 1 = 1-5 tiller, 2 = 6-20 tillers, 3 = 21-50 tillers, 4 = 51-90 tillers and 5 = > 90 tillers. An average for each population was then calculated prior to statistical analysis. Plant type was also estimated using a categorical 1-3 scale where, 1 = bunch, tufted, erect or non-spreading, 2 = pseudo erect, not strongly bunched, tufted nor decumbent and 3 = spreading, decumbent or prostrate. All observations were made 91 days after transplanting (180 days after seeding).

The recovered interspecific and intergeneric ASR368 species RR hybrids (ASR368 x non-transgenic hybrid) were either equivalent to or considerably less robust than their respective maternal and paternal parent species collections with regard to ground cover, growth habit or tillering capacity (Table VII.C.5).

In 2002, these same hybrids were evaluated in the field to confirm that the recovered ASR368 x non-transgenic hybrids remained comparable to or less robust than their respective maternal and parental species with respect to growth rate. Vegetative tillers of each hybrid and parent were transplanted in the field into cultivated soil on August 15 and 16, 2002 in Marion County, Oregon. Each transgenic hybrid was represented by four replications of three or four plants arranged in a randomized complete block design. Soil fertility and moisture were maximized to ensure an optimum growing environment throughout the study. The diameter of each plant canopy was recorded on October 13, 2002 as a measurement of plant diameter (cm). Plant diameter was then transformed to percent ground cover based on the field space provided per individual plant.

The plant diameter and percent ground covered by the ASR368 x non-transgenic hybrids were either not significantly different from or smaller than their related non-transgenic parent species (Table VII.C.6).

After two years of studying these hybrids, the results further demonstrate that were hybrid seed set and seedling establishment to occur, it is unlikely those hybrids would be any more invasive than plants that exist among the *Agrostis* and *Polypogon* genera today.

C.4. Overall conclusions of event ASR368 transgene flow studies

The results of these studies are consistent with the findings of researchers discussed previously: (1) that creeping bentgrass can form intraspecific, interspecific and intergeneric hybrids with several other *Agrostis spp*. and *Polypogon spp*.; (2) outcrossing frequency falls precipitously as distance increases from the pollen source; (3) that prevailing wind influences the frequency of gene flow and (4) the vegetative growth and plant growth habit of the recovered hybrids were either intermediate or less than their two parent species.

The results also demonstrate that although event ASR368 may form intraspecific *A. stolonifera* RR hybrids up to 354 m in the direction of the prevailing wind, there is reduced likelihood that interspecific or intergeneric RR hybrid formation will occur as distances increase beyond 50 meters. Further, the frequency of hybridization at the farthest distances is very low (~ 0.1%), yet may still overestimate the potential for gene flow in a seed production field since isolated or small populations of pollen receptor plants are more apt to be pollinated by their nearest neighbor than a remote pollen source (Griffiths, 1951 and Knowles, 1966).

The data from this study further support the use of spatial isolation to maintain the varietal purity of conventional and transgenic creeping bentgrasses. Spatial isolation and other cultural management practices have been effectively implemented in the production of high quality creeping bentgrass and other turf seeds for more than 75 years and should continue to do so into the future.

It is important to note that although hybrids with *A. stolonifera* and event ASR368 may form, all evidence to date suggest that a hybrid with the Roundup Ready trait would not provide any potential increased risk of weediness as its growth habit would be similar to its parents. In addition, as discussed in Section II.F.4.e, the persistence of any hybrid offspring through sexual reproduction is unlikely due to the general sterility of F1 hybrids. However, even in the rare case of a fertile F1 hybrid, the fitness of the F2 hybrids is highly questionable (Bradshaw, 1958b).

Finally, multiple options are available for the control of any potential Roundup Ready creeping bentgrass hybrids that would form, including physical methods or the use of non-glyphosate based herbicides as such as fluazifop, sethoxydim and clethodim, as discussed in the following section and Appendices VI and VII.

Figure VII.C.1. a and b. Plot design for pollen-mediated intraspecific, interspecific and intergeneric transgene flow study conducted in Franklin County, WA from 2001 and 2002.

Figure VII.C.1.a. 2001

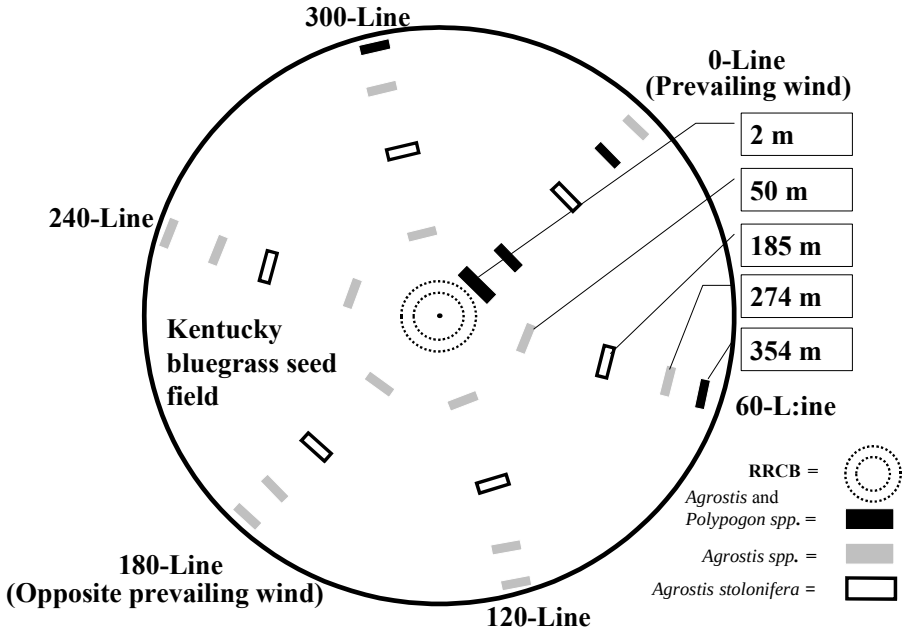

Figure VII.C.1.b. 2002

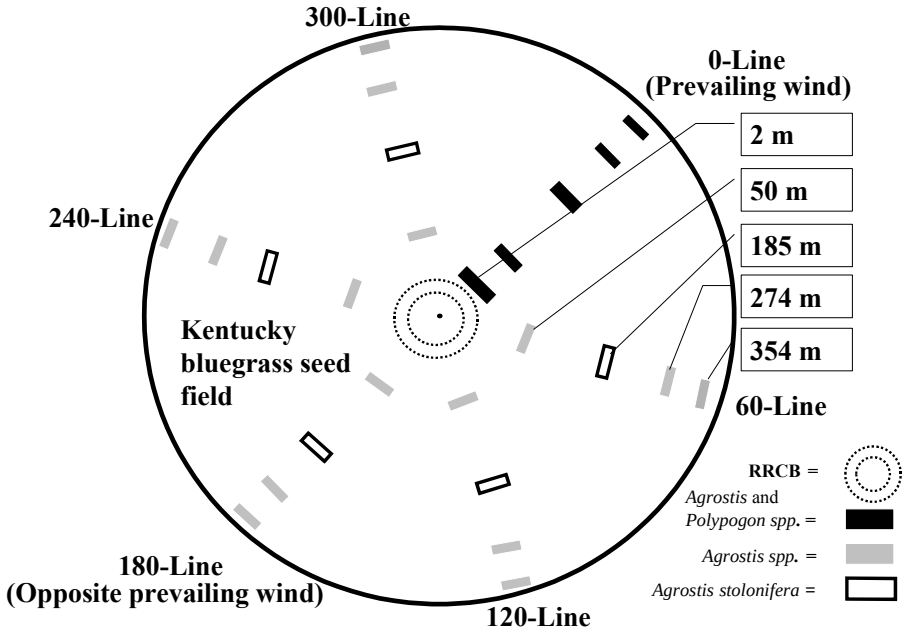

Table VII.C.1.a. Percentage of event ASR368 intraspecific *A. stolonifera* progeny recovered from plants at different line and distance plots in 2001 at Franklin County, WA.

		2001				
		Distance (m) from ASR368 pollen (transgene) donor				
		2[1]	50	185	274	354
Line	0[2]	48.87	0.56	0.38	0.15	0.07
	60		0.27	0.03	0	<0.01
	120		0.02	0	0	0
	180		0[3]	0	0	0
	240		0.07	0	0	0
	300		0.59	0.03	0.03	0.03

[1] Only a single plot was planted 2 m from the RRCB transgene donors (0-line 2-m plot).
[2] The 0-line was aligned with the prevailing wind direction. All other lines indicate compass degrees clockwise from the 0-line.
[3] All receptor *A. stolonifera* died following glyphosate application by a custom applicator.

Table VII.C.1.b. Percentage of event ASR368 intraspecific *A. stolonifera* progeny recovered from plants at different line and distance plots in 2002 at Franklin County, WA.

		2002				
		Distance (m) from ASR368 pollen (transgene) donor				
		2[1]	50	185	274	354
Line	0[2]	26.63	0.94	0.19	0.02	0.01
	60		2.74	0.16	0.11	0.15
	120		0.85	0.02	0.01	0
	180		0.75	<0.01	<0.01	0
	240		0.08	<0.01	0	0
	300		0.39	0.01	<0.01	0

[1] The plot 2 m from the RRCB transgene donors was only present on the 0-line (0-line 2-m plot).
[2] The 0-line was aligned with the prevailing wind direction. All other lines indicate compass degrees clockwise from the 0-line.

Table VII.C.2. Hybrid formation between event ASR368 and related species placed 1 to 3 m along the direction of the prevailing wind from event ASR368 (pollen donor) during 2001 and 2002 at Franklin County, WA.

	2001			2002		
Species	**n[1]**	**RR progeny recovery (%)**	**Std. dev.**	**n**	**RR progeny recovery (%)**	**Std. dev.**
A. stolonifera	7	48.87	22.29	11	26.63	13.85
A. trinii	2	10.56[2]	13.34	1	0	0
P. monspeliensis	1	1.58	0	4	0.74	0.45
A. pallida	3	1.45	2.32	*	*	*
P. fugax	3	1.42	1.01	2	2.68	1.01
A. idahoensis	5	0.8	1.01	5	0.39	0.53
P. viridis	3	0.69	0.22	2	1.3	0.93
A. pallens	4	0.52	0.01	6	0.01	0.03
A. capillaris	5	0.37	0.55	10	0.4	0.51
A. gigantea	8	0.05	0.04	8	0.17	0.18
A. canina	5	0.03	0.16	4	0.11	0.23
A. vinealis	6	0.01	0.03	*	*	*
A. hyemalis	5	0.002	0.006	*	*	*
A. castellana	5	0	0	10	0	0
A. nebulosa	5	0	0	*	*	*
Apera interrupta	*	*	*	5	0	0

[1] Number of subsamples for each species. The percentage RR progeny recovery for each subsample (plant) was determined and then a mean percentage RR progeny recovery calculated across subsamples.

[2] Species identity of single plant harvested as *A. trinii* is considered to be a creeping bentgrass contaminant that perished following pre-emergent herbicide application following the first year harvest.

* Species was not included during this year of the study.

Table VII.C.3. Hybrid formation between event ASR368 and related species placed 50 m along the direction of the prevailing wind (0^0 axis) from event ASR368 (pollen donor) during 2001 and 2002 at Franklin County, WA.

Species	% RR Progeny Recovered	
	2001	2002
P. fugax	0.006	0.021
P. monspeliensis	0.00	0.017
P. viridis	0.023	0.012
A. capillaris	0.026	0.011
A. castellana	0.000	0.000
A. gigantea	0.000	0.008
A. hyemalis	0.005	np
A. idahoensis (0^0 axis)	0.017	0.000
A. idahoensis (60^0 axis)	0.000	0.004
A. idahoensis (300^0 axis)	0.000	0.003
A. idahoensis (300^0 axis)	0.000	0.003
A. pallens	0.000	0.000

np not planted in 2002

Table VII.C.4. Conventional (non-transgenic) parent species used as reference comparisons for event ASR368 hybrids of the same parent species in 2001 and 2002.

Scientific Name	Common Name	Plant Type Hybrid or Conventional*
Agrostis stolonifera L.	Creeping Bentgrass	C
Agrostis capillaris L.	Colonial Bentgrass	C, H
Agrostis gigantea Roth.	Redtop Bentgrass	C, H
Agrostis idahoensis Nash.	Idaho Bentgrass	C, H
Agrostis pallens	Dunes Bentgrass	C, H
Agrostis trinii Turcz.	Brown Bentgrass	C, H
Polypogon monspeliensis L. Desf.	Rabbitsfootgrass	C, H
Polypogon viridis (Gouan) Breistr.	Watergrass	C, H
Polypogon fugax Nees es Steud.	Ditch *Polypogon*	C, H
Agrostis castellana Boiss.& Reut.	Dryland Bentgrass	C,
Agrostis canina L.	Velvet Bentgrass	C,
Agrostis vinealis Schreb.	Brown Bentgrass	C,
Agrostis sp.	Rhode Island Bent	C,

* Where C = conventional and H = hybrid formed between RRCB event ASR368 and conventional

Table VII.C.5. Plant characteristics of hybrids recovered from event ASR368 and related species in 2001.

Plant Population[1]	Percent Ground Cover[2]	Tillers/Plant (1-5[3])					Growth Habit (1-3[4])		
		% Occurrence per Rating Unit							
		1	2	3	4	5	1	2	3
A. stolonifera (Creeping Bent) Conventional	20.47 d	0	17	67	17	0	44	27	27
A. capillaris (Colonial Bent) Hybrid	14.33 h-k	0	33	67	0	0	67	33	0
A. capillaris (Colonial Bent) Conventional	15.50 e-h	0	33	44	22	0	56	11	33
A. gigantea (Redtop) Hybrid	15.00 f-i	0	50	50	0	0	75	17	8
A. gigantea (Redtop) Conventional	10.65 jkl	22	44	33	0	0	83	17	0
A. idahoensis (Idaho Bent) Hybrid	9.43 l	11	56	22	11	0	67	33	0
A. idahoensis (Idaho Bent) Conventional	14.53 g-j	0	44	44	11	0	22	22	56
A. pallens (Dunes Bent) Hybrid	10.75 jkl	0	58	33	8	0	67	17	17
A. pallens (Dunes Bent) Conventional	29.33 a	0	0	67	33	0	33	67	0
P. monspeliensis (Rabbitsfootgrass) Hybrid	19.43 de	0	0	67	33	0	78	22	0
P. monspeliensis (Rabbitsfootgrass) Conventional	18.30 d-g	22	44	22	11	0	89	11	0
P. fugax Hybrid	20.13 d	0	44	56	0	0	100	0	0
P. fugax Conventional	21.80 cd	0	56	33	11	0	89	11	0
P. viridis (Watergrass) Hybrid	15.23 f-i	0	67	33	0	0	100	0	0
P. viridis (Watergrass) Conventional	25.13 bc	0	67	22	11	0	22	78	0
A. castellana (Dryland Bent) Conventional	28.37 ab	6	17	39	39	0	50	50	0
A. canina (Velvet Bent) Conventional	18.57 def	0	22	44	22	11	44	22	33
A. trinii Hybrid (Brown Bent)	5.33 m	36	64	0	0	0	100	0	0
A. trinii Conventional	10.40 kl	0	89	11	0	0	100	0	0
A. vinealis (Brown Bent) Conventional	10.50 kl	0	44	56	0	0	67	22	11
A. sp. (Rhode Island Bent) Conventional	19.53 d	0	44	44	11	0	67	33	0
LSD (p=0.05%)	3.964								
Stn. Deviation	3.433								

[1] Conventional or hybrid formed between RRCB event ASR368 and conventional
[2] Means followed by the same letter are not significantly different (LSD, p = 0.05)
[3] Tillers/plant: 1 = 1 to 5; 2 = 6 to 20; 3 = 21 to 50; 4 = 51 to 90; 5 = greater than 90
[4] Growth habit: 1 = bunch, tufted, erect, not spreading; 2 = pseudo-erect, not strongly bunched, tufted or decumbent; 3 = spreading, decumbent, prostrate

Table VII.C.6. Plant characteristics in 2002 of conventional *Agrostis* and *Polypogon* species used as comparators and interspecific and intergeneric RR hybrids with creeping bentgrass event ASR368 recovered from related *Agrostis* and *Polypogon* species in 2001.

Plant Population	Relationship	n	Plant Diameter (cm)[2]	Ground Cover (%)[3,4]
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. capillaris (Colonial Bent) Hybrid	Hybrid	12	33.3fgh	57.1def
A. capillaris (Colonial) Conventional	Parent	12	38.7def	75.5def
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. gigantea (Redtop) Hybrid	Hybrid	16	39.6def	78.6de
A. gigantea (Redtop) Conventional	Parent	20	44.5cde	103.1cd
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. idahoensis (Idaho Bent) Hybrid	Hybrid	12	24.3hi	31.5ef
A. idahoensis (Idaho Bent) Conventional	Parent	12	23.4hi	27.9ef
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. pallens (Dunes Bent) Hybrid	Hybrid	16	36.1efg	63.9def
A. pallens (Dunes Bent) Conventional	Parent	12	39.5def	76.4def
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
P. monspeliensis (Rabbitsfootgrass) Hybrid	Hybrid	12	39.9def	77.5de
P. monspeliensis (Rabbitsfootgrass) Conventional	Parent	12	58.5ab	187.3b
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
P. fugax Hybrid	Hybrid	12	40.1def	84.9de
P. fugax Conventional	Parent	12	67.9a	267.2a
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
P. viridis (Watergrass) Hybrid	Hybrid	12	47.3cd	109.5cd
P. viridis (Watergrass) Conventional	Parent	12	53.2bc	147.bc
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. trinii Hybrid (Brown Bent)	Hybrid	16	35.9efg	78.7de
A. trinii Conventional	Parent	12	25.3ghi	31.5ef
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. pallida	Hybrid	16	36.6def	77.8de
LSD (p=0.05%)			11.17	60.44
Std Deviation			7.9	42.74

[1] conventional or hybrid formed between RRCB event ASR368 and conventional
[2] Means followed by same letter are not significantly different (LSD, p = 0.05)
[3] Means followed by same letter are not significantly different (LSD, p = 0.05)
[4] Percent ground cover based on plant spacing of 45.7cm (1.5 ft)

D. Control of event ASR368 and conventional bentgrasses with post-emergent herbicides

The development and potential commercialization of glyphosate tolerant creeping bentgrass requires the identification of pest management measures and alternative herbicides where it is desired to control glyphosate resistant plants.

Mechanical removal of bentgrass patches by scalping with mowers and reseeding or by cutting out the sod and replacing with new sod are reported as effective options for bentgrass control in golf course roughs planted to other species that adjoin creeping bentgrass fairways (Snow, 1982; Huber, personal communication).

Herbicides in the cyclohexanedione and aryloxyphenoxy propionate families are commonly used to control both annual and perennial grasses in a wide variety of dicot agronomic crops (Ahrens 1994). Several of these herbicides such as fluazifop, clethodim and sethoxydim are also labeled for broadcast or directed applications in landscape planting beds and some turfgrass species for removal of unwanted annual and perennial grass species (BASF Corp. and MicroFlo Co., 2002; Valent USA Corp., 2002; Zeneca Ag Products, 2001). Glyphosate controls plants by inhibiting the enzyme EPSP synthase involved in the synthesis of aromatic amino acids (Amrhein, *et al* 1980). Cyclohexanedione and arlyoxyphenoxy propionate herbicides control grass species by inhibiting the enzyme acetyl-CoA carboxylase (ACCase) that is involved in fatty acid biosynthesis (Burton et. al., 1989; Focke and Lichtenthaler, 1987). Another herbicide alternative that has potential for control of glyphosate tolerant creeping bentgrass and related species is glufosinate, which is a non-selective herbicide that controls plants by inhibiting the enzyme glutamine synthase, resulting in a toxic accumulation of ammonia in plant cells (Logusch et al., 1991; Wild et al., 1987; Wild and Wendler, 1993).

There is little information currently available on the response of creeping bentgrass and related bentgrass species to applications of the cyclohexanedione herbicides such as clethodim and sethoxydim and the aryloxyphenoxy propionate herbicide fluazifop (Rely 24(c) label; UC Turf Sensitivity Guideline, 1997; Nova Scotia, Canada, Environmental Horticulture Dept.). Cook *et al.* (1996) conducted a study that examined efficacy of glufosinate and glyphosate to control colonial bentgrass (*Agrostis capillaris* L.) turf in Oregon. Cook reported that 42 days after treatment, both products provided similar and complete control of the bentgrass. The objectives of this research conducted in 2001 and 2002, which is summarized here and attached in its entirety in Appendices VI and VII, were to: (1) evaluate the potential for these herbicides to control glyphosate tolerant and susceptible creeping bentgrass, as well as colonial, redtop, and dryland bentgrass in individual plant stands, (2) evaluate the parallel utility of these products to control creeping bentgrass growing as a sod (3) further define the effects of plant age on herbicide susceptibility and (4) evaluate the potential for these herbicides to control hybrids that may form between ASR368 and related species.

D.1. Potential for alternative herbicides to control glyphosate tolerant and susceptible creeping bentgrasses in individual plant stands

Experiments to evaluate control of individual bentgrass plants were conducted in 2001 at Rutgers University in North Brunswick, New Jersey and at the Scotts Company Research farm located in Marion County, Oregon.

Bentgrass plugs were transplanted into bare ground on May 9 and August 7, 2001 in New Jersey and on May 21 and August 16, 2001 in Oregon. The bentgrass species evaluated included: event ASR368 Roundup Tolerant (RR); event ASR368 Roundup Susceptible (RS); a mixture of commercial creeping bentgrass hybrids ('Penn A-4', 'Backspin' and 'Crenshaw'); colonial bentgrass (*Agrostis capillaris* L. 'SR 7100'); redtop bentgrass (*Agrostis gigantea* With. 'Streaker'); and dryland bentgrass (*Agrostis castellana* Boiss. and Reut.'Trust').

The results of these studies demonstrated that glufosinate and the ACCase inhibiting herbicides fluazifop, clethodim and sethoxydim have substantial herbicidal activity on creeping bentgrass and related species (Appendix VI). No differential response between RR phenotype and RS phenotypes of creeping bentgrass to glufosinate or the ACCase inhibiting herbicides was observed in these experiments, confirming that, as in other crop species, the *cp4 epsps* gene conferring glyphosate resistance does not increase tolerance to herbicides that do not inhibit the EPSP synthase enzyme (Appendix VI).

Control of glyphosate susceptible bentgrass with glyphosate was nearly complete in all experiments. Bentgrass species are generally considered to have a high level of tolerance to glyphosate relative to other perennial grass species as evidenced by the listing of only partial control on many glyphosate herbicide labels (Monsanto Co., 2002). The high levels of control of *Agrostis spp.* that Cook, *et al.* (1996) observed by glyphosate as well as the other herbicides may be indicative of the treatments applied to individual bentgrass plants, leading to maximum herbicide contact with the bentgrass foliage and exposed stolons, such as would be encountered with spot treatment in seed production or landscape beds.

In summary, fluazifop appears to have potential to be utilized as an herbicide alternative for control of glyphosate tolerant bentgrass plants growing individually in areas where they are undesirable. Glufosinate and clethodim also demonstrated substantial herbicidal activity on the bentgrass species evaluated.

D.2. Potential utility of alternate herbicides to control creeping bentgrass growing as sod

In view of the preceding results that RR and RS phenotypes of bentgrass were equally susceptible to alternative herbicide treatments, further research was conducted to assess the utility of the ACCase inhibitor herbicides in controlling mature sods of RS phenotypes of creeping bentgrass. Experiments were conducted at Purdue University at West Lafayette, Indiana and North Carolina State University at Jackson Springs, North

Carolina. Creeping bentgrass sod was established in fall, 2000 in Indiana using the cultivar, Providence. Sod was established in 1995 using the cultivar, Penncross, in North Carolina.

The results of studies on creeping bentgrass sod demonstrate that it represents a more challenging weed control target than individual plants for both glyphosate and alternative herbicides (Appendix VI). Whereas results indicate that acceptable levels of control could be achieved with single applications of ACCase inhibitor herbicides on individual plants, sequential applications were needed to provide optimal results on sod. Similarly, glyphosate provided good control with single plants but was inconsistent for controlling sod, consistent with label claims for this product (Appendix VI).

These results should be considered carefully, however. Under normal practice, tillage would be utilized in addition to herbicides to destroy creeping bentgrass sod in either a golf fairway/green, sod farm or seed production field. Consequently, tillage represents a substantial asset when combined with an effective herbicide program for eliminating any remaining vegetation not killed by the herbicide.

The improved activity found with individual plants from a fall ACCase inhibitor application suggests that sod removal may be improved further through use of a fall-timed application.

In conclusion, control of either non-transgenic creeping bentgrass or Roundup tolerant creeping bentgrass is possible with several herbicides in addition to those based on glyphosate. A combination treatment of glyphosate and fluazifop or clethodim appears to be compatible for use in site renovation to control mixed populations of glyphosate tolerant and susceptible bentgrass, annual bluegrass, and broadleaf weed species.

D.3. Effects of plant age on herbicide susceptibility

The results of alternative control measures with individual plants and with sods suggest that several herbicides can be employed to control Roundup Ready creeping bentgrass. During 2002 additional studies were conducted to both confirm the results from previous years and further understand the effect of sod age on control level of RS phenotypes. Experiments were conducted during 2002 at Purdue University (two trials) at West Lafayette, Indiana, North Carolina State University (two trials) at Jackson Springs, or Carolina, the University of Massachusetts at Amherst, Massachusetts and Washington State University at Puyallup, Washington. Creeping bentgrass sod was established in 1995 in Indiana, in 1998 in North Carolina, the “early ‘90’s” in Massachusetts and in the “mid ‘90s” in Washington.

The results of the 2002 studies confirm that on older sod and sod under drought stress, the performance of a single application of glyphosate varies on creeping bentgrass (Appendix VI). Under these stress conditions, the ACCase inhibitor herbicides may also show less control. Clethodim generally provided the best control under these conditions and appears to be comparable to or better than glyphosate under a drought scenario. The

need for a sequential application of an ACCase herbicide to control a difficult species like creeping bentgrass is obvious from both 2001 and 2002 studies. Under drought stress a light tillage should be recommended after complete translocation of any of these products to provide complete control. Fluazifop and sethoxydim, also show potential for creeping bentgrass control. However, in older sod, they would benefit from a light tillage or standard sod removal following the second application to provide optimal results (Appendix VI).

D.4. Mitigation of ASR368 and intra-specific, inter-specific and inter-generic hybrids

An experiment to evaluate the control of event ASR368 intra-specific, inter-specific and inter-generic hybrids grown as individual plants was conducted in the poly-house at Marion County, Oregon, during the spring and fall of 2002. Hybrids were derived either from forced crosses in the greenhouse or from seed obtained in field experiments (Christoffer 2003, Appendix VII).

Hybrids included in this trial were crosses of Roundup Ready creeping bentgrass (*Agrostis stolonifera*) with *A gigantea, A. idahoensis, A. pallens, A. capillaris, Polypogon monspeliensis, P. fugax* and *P. viridis*. One to four hybrid accessions were examined. These accessions were compared within the experiment for control with their respective parents where possible (event ASR368 and the above compatible species). Also included for comparison were the susceptible commercial creeping bentgrass cultivars Southshore, Crenshaw, Backspin, Penneagle and Penn A-4.

Consistent with the previously discussed studies, effective herbicide solutions were identified in these experiments to address the need to control hybrids that may form on rare occasion from pollen flow to sexually compatible relatives from Roundup Ready creeping bentgrass (Appendix VII). The solution of choice may differ depending on environmental conditions characteristic of seasonal variation. Multiple active ingredients are available (ACCase inhibitor herbicides or glufosinate) that via either single or multiple applications can remove these plants as effectively as glyphosate can remove their non-transgenic predecessors.

D.5. Conclusions from alternate herbicide trials

These studies demonstrated that there are several alternative herbicides that can control creeping bentgrass or its hybrids with related species growing either as individual plants or in a sod (Appendices VI and VII). The use of these materials in tank mixes with glyphosate will provide broad-spectrum weed control including control of Roundup Ready creeping bentgrass. The effect of these mixtures will provide solutions that are equivalent to or better than those that were known before the advent of Roundup Ready creeping bentgrass. The ACCase inhibitor class of chemistry is large (Tomlin, 1997) and includes many commercial products that have activity on creeping bentgrass. These studies have focused on herbicides that are already labeled for key target sites and could

be available for immediate use by the golf and grass seed production industries. Results from 2001 and 2002 show the age of sod and environmental conditions have no greater effect on the performance of alternative herbicides for the control of creeping bentgrass than they do on glyphosate (Appendices VI and VII).

E. Stewardship implications for Roundup Ready creeping bentgrass

E.1. Introduction

As discussed in Section II of this petition, creeping bentgrass is characterized as a low-stature, fine-textured, soft, very dense, carpet-like turfgrass sward that tolerates low mowing. When used as a close-mowed turf, frequent watering, optimum fertilization, disease management and soil management practices are needed to prevent competition from other grass or broad-leafed weed species. Even under optimal nutrition and watering regimes, creeping bentgrass is susceptible to a wide range of diseases, including pink snow mold, brown patch and dollar spot. Therefore, because of the intense level of management required, creeping bentgrass is rarely employed for residential use but rather for golf course putting greens, tees and fairways, lawn bowling greens, grass tennis courts and other specialized applications. Monsanto and The Scotts Company intend to market Roundup Ready creeping bentgrass exclusively to the golf course market through seed and sod production. There is no intention for this product to be sold for residential, industrial or other recreational applications.

Monsanto and The Scotts Company have developed a comprehensive stewardship plan to be implemented by all users of this technology, and the specialized but highly valued end use of Roundup Ready creeping bentgrass will help facilitate effective implementation of that plan. In order to meet the current total demand for creeping bentgrass seed used by United States golf courses, only 7,000 acres are farmed annually for seed production and ca. 95% of this acreage is planted in the Willamette Valley of Oregon. To satisfy the anticipated demand by sod farms and golf courses for Roundup Ready creeping bentgrass seed, it is anticipated that considerably less acreage, perhaps no more than 3,000 acres, would be needed. This amount of seed can be grown by less than two-dozen contracted seed producers with sufficient isolation to essentially prevent outcrossing to or from conventional creeping bentgrass seed production farms. Roundup Ready creeping bentgrass seed will also be combined and cleaned using equipment dedicated to the crop. Seed will be shipped directly to the sod farm or golf course to further ensure its use only by these customers.

Turf grass sod is often used in order to establish golf course fairways and putting greens more quickly to both limit erosion and reduce the time the course is closed for golf play while the turf is establishing. Sod farms maintain the height of cut at the same level as golf course fairways and/or putting greens. As discussed earlier, at these low cutting heights, creeping bentgrass is unlikely to flower, pollinate nor produce mature seed. Shipment of cut sod to golf courses will occur via covered trucks directly from the sod farm.

Golf courses are managed and maintained by professional superintendents and their staff. The relatively few number of course employees involved will enable direct and frequent communications regarding stewardship and cultural practices. Monsanto and The Scotts Company will use these practices to deter potential outcrossing and environmental persistence and the potential development of weed resistance to Roundup Pro herbicide. Therefore, this section reviews the stewardship practices in development by Monsanto and The Scotts Company for each component of the channel from seed and sod production to the ultimate end use on golf courses.

E.2. Seed production of Roundup Ready creeping bentgrass

At least 95% of creeping bentgrass seed production occurs in the Willamette Valley of Oregon. United States seed production annually ranges between 4,500 and 7,000 acres for use on golf courses and less frequently on croquet and tennis courts and other sporting fields requiring very close-cropped turf. Most of this seed is sold domestically, but importers in several other countries including Canada, the European Union and Japan also purchase creeping bentgrass seed from U.S. producers.

To reduce the potential for outcrossing to or from conventional creeping bentgrass varieties for domestic or export use, Monsanto and The Scotts Company intend to produce Roundup Ready creeping bentgrass in geographically isolated areas, which provide sufficient spatial separation from conventional creeping bentgrass. In addition, seed growers and seed processors producing and finishing Roundup Ready creeping bentgrass seed will be required by contract to follow production practices that include the following:

(1) Roundup Ready creeping bentgrass seed must be combined with equipment dedicated to Roundup Ready creeping bentgrass.

(2) Roundup Ready creeping bentgrass seed must be transported from the field to the cleaning and packaging facility in enclosed containers.

(3) Roundup Ready creeping bentgrass seed must be processed at a seed cleaning and packaging facility in which no other turfgrass seed crops shall be cleaned or packaged.

(4) Roundup Ready creeping bentgrass seed will be packaged in leak resistant containers.

(5) Routine monitoring of vegetation surrounding the production area to scout for and devitalize or mow (to prevent pollination or seed production) Roundup Ready creeping bentgrass volunteers or hybrids with related species.

After the appropriate regulatory clearances have been received and Roundup Ready creeping bentgrass is commercialized, seed will be produced on relatively few acres (probably < 3,000) to meet the demand of the golf course industry. It is expected that no

more than two dozen growers will be needed to produce this amount of seed. Consequently, the ability to regularly monitor, communicate and educate Roundup Ready creeping bentgrass seed producers on stewardship requirements through farm visits, grower meetings, mailings, etc. will be greatly facilitated, as will the opportunity to identify and rectify any concerns that may arise.

Finally, it is important to note that the adventitious presence of the *cp4 epsps* gene in conventional creeping bentgrass or species with which *A. stolonifera* can interbreed would pose no environmental risk because glyphosate tolerance confers no competitive advantage. Further, mechanical controls and several other grass herbicides such as fluazifop, sethoxydim, and clethodim are equally or more effective at controlling bentgrass than glyphosate (Section VII.D and Appendices VI and VII).

E.3. Harvest of Roundup Ready creeping bentgrass seed

As discussed in Section II of this petition, creeping bentgrass is usually not a contaminant in other turf grass species because of its relatively late maturity date. Creeping bentgrass, including Roundup Ready creeping bentgrass, would be the last grass to mature and, consequently, the last grass harvested in the grass seed production areas of Oregon, Washington and Idaho. Just prior to harvest, fields are swathed and the seed is allowed to dry in the field before combining to separate seed from straw. Perennial ryegrass, roughstalk bluegrass, Kentucky bluegrass and other grass species are harvested three to four weeks earlier when most creeping bentgrass seed is still immature. Nonetheless, a dedicated combine thresher will be used to harvest Roundup Ready creeping bentgrass seed to avoid mixing, which is the likely mechanism by which Roundup Ready creeping bentgrass (or conventional bentgrass) might be included in other turf grass seed lots.

E.4. Cleaning and distribution of Roundup Ready creeping bentgrass seed

At 0.07 mg seed^{-1}, creeping bentgrass seed is among the smallest of the grass seeds commercially produced. Consequently, it is easily separated from leaf material and most other grass or weed seeds by passing through a series of seed cleaners, indent cylinders, Carter disc separators, and gravity tables. The cleaned seed may then be further treated with a fungicide prior to packaging in bags or sealed plastic containers. The packaged seed is best stored in cool and/or dry conditions prior to ultimate distribution to the end user. The plant material separated from the seed or "seed screenings" are burned, composted or pelleted for sale as a supplemental animal feed.

To enable the use of Roundup Ready creeping bentgrass seed screenings as a supplemental animal feed, Monsanto and The Scotts Company have been consulting with the U.S. Food and Drug Administration since October 2001 per their policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, published in the Federal Register on May 29, 1992. A summary of the feed safety and nutritional assessment of event ASR368 was provided to the Agency on September 13, 2002.

The current distribution system for creeping bentgrass seed entails shipment via truck and/or rail to resellers or wholesalers who often store the seed in their own warehouses prior to shipment to a regional distributor. From there the seed is shipped to the end user, which is typically either a golf course or a sod farm.

As the size of Roundup Ready creeping bentgrass and conventional creeping bentgrass seed are expected to be the same, the cleaning process will not change. Only sealed plastic containers will be used to package Roundup Ready creeping bentgrass seed to reduce or eliminate the potential for accidental seed scatter during distribution to the end user through punctured or ripped bags.

Direct distribution from the seed-conditioning warehouse to the golf course or sod farm will be implemented to further reduce the potential for seed to inadvertently scatter or to reach unintended markets. The distribution system for Roundup Ready creeping bentgrass seed will not involve physical shipment through the standard grass seed distribution channel described above. Rather, seed will move directly from the dedicated seed-conditioning warehouse to the golf course or sod farm.

E.5. Sod farm and golf course stewardship of Roundup Ready creeping bentgrass

Roundup Ready creeping bentgrass will only be sold for use on sod farms for golf courses use. Therefore, this section will discuss the stewardship practices in development for sod farms and golf courses to limit the unintentional spread of Roundup Ready creeping bentgrass.

Like golf courses, sod farms establish turf through the planting of seed. The growing turf is then maintained at the same height as its intended use i.e., golf course tees, fairways and/or putting greens. As discussed earlier, creeping bentgrass is unlikely to flower, pollinate or set mature seed under these management conditions (Lush, 1988). Harvested sod is cut as either slabs or rolls, palletized and then loaded onto trucks to be shipped directly to local golf courses. Cut sod is perishable and therefore is typically produced and shipped rapidly to golf course customers.

The Scotts Company and Monsanto will only ship Roundup Ready creeping bentgrass to licensed sod producers to grow Roundup Ready creeping bentgrass sod. The sod will be sold only for use on licensed golf courses and will be shipped only in enclosed or covered trucks for use on those golf courses. Both sod farms and golf courses manage the planting and maintenance of turf in the same manner. Consequently, although the following discussion will focus on golf courses, these same stewardship practices will apply to sod farms as well.

Creeping bentgrass has been planted as a playing surface on golf course greens, tees, and fairways in cool season turfgrass growing areas of North America for over 100 years (Duich, 1985; Hurley and Murphy, 1996). The tolerance of creeping bentgrass to extremely close mowing and its stoloniferous spreading habit makes it especially well adapted for use as a golf course turf (Reese, 2000). Creeping bentgrass excels at mowing

heights between 1/8 to 3/4 inches that are common to golf course greens and fairways. At these heights the turf is unlikely to either pollinate, flower or produce mature seeds that are disruptive to play or can spread the turf to undesired areas of the course (Lush, 1988). In addition, its hardy vegetative growth response to water, nitrogen, fertilizer and sunlight allows the turf to recover from the damages of continuous traffic and disturbance from golf clubs.

When establishing a new golf course or replacing existing turf on a fairway or green, course managers strive for the most rapid germination and grow-in possible, so that the course is suitable for play as quickly as possible. Creeping bentgrass seeds are extremely small and therefore, limited in carbohydrate energy reserves. Consequently, successful establishment requires a highly prepared seedbed and ample irrigation and fertilizer to encourage germination and rooting. Once established, maintenance of the turf for both play and aesthetics requires regular mowing two to seven days a week as well as daily irrigation and routine fertilizer and soil management inputs. Such intensive management makes creeping bentgrass undesirable for use on residential or industrial lawns (Turgeon, 2002).

The Scotts Company, Monsanto and/or their designates will monitor golf courses and sod farms who will be responsible for implementing these stewardship practices. The goal of this aspect of the stewardship plan will be to ensure to the extent possible that Roundup Ready creeping bentgrass plant material remains on the golf course and sod farms. Areas identified for Roundup Ready creeping bentgrass stewardship practices by golf course superintendents focus on: (1) implementing precautionary measures that minimize, to the extent possible, potential seed and stolon scatter or movement via equipment of this plant material to other golf courses or sod farms; (2) maintaining Roundup Ready creeping bentgrass turf on greens, tees and fairways at playing heights that would preclude pollination, flowering and mature seed head development; (3) devitalizing stolons and aerification cores that could enable unintentional vegetative propagation to occur; and (4) controlling volunteers surviving from the renovation or abandonment of a golf course or from the sod farm after harvest of Roundup Ready creeping bentgrass sod.

The exacting playing and aesthetic conditions required by golfers, course members and owners necessitates that course superintendents are experienced and well educated in their discipline. Consequently, most course superintendents are well-trained professionals, holding a degree in horticulture or a similar plant science field. In addition to the superintendent, often a staff of more than a dozen individuals assists in maintaining the course. Monsanto and The Scotts Company will provide training, recommendations and guidelines for the above stewardship practices in their published Technical Use Guide, which will accompany the license agreement for the growth and use of Roundup Ready creeping bentgrass. As an alternative, the golf course or sod farm may develop their own best practices for performing the above responsibilities compatible with their own course or farm conditions. The Scotts Company and/or Monsanto will review these stewardship plans. In either case, these guidelines will be communicated before a license to the technology is issued to the golf course. These stewardship practices will be

included in the contractual agreement governing the use of the Roundup Ready creeping bentgrass seed.

E.5.a. Equipment maintenance

It is not uncommon for mowers, seeders, aerifiers and other equipment used to maintain golf course turf to be shared between courses. Golf course superintendents and those contracted for the construction or establishment of Roundup Ready creeping bentgrass will be responsible for minimizing, to the extent possible, seed or viable stolon transport between courses. Consequently, this responsibility will include the cleaning and inspection of equipment to ensure proper sanitation has been achieved before turf maintenance equipment leaves the course. For sod farms, this same principal applies when the equipment is used on sod fields of other turfgrass species or varieties.

E.5.b. Devitalization of stolons and aerification cores

Maintaining the appropriate clipping height is a criterion for playability and is therefore, not a stewardship concern. However, the mown grass clippings may contain stolon nodes, which, if they come in contact with the soil, may root and continue to grow. Likewise aerification cores that contain stolons and roots in a plug of soil may also continue to grow in unintended areas. Although vegetative growth from either stolons or aerification cores is rare on a golf course or sod farm because of the intense manner in which the turf is managed, this plant material will either be: (1) composted in designated areas of the course and monitored for regrowth and devitalized; (2) monitored for regrowth where deposited and devitalized or (3) managed in an appropriate way to ensure undesired regrowth does not occur. Devitalization of surviving plant material can be accomplished through mechanical means or treatment with one of the several effective herbicides, including fluazifop, sethoxydim and clethodim.

E.5.c. Redesigned or abandoned golf courses

Golf courses may be redesigned to improve or modify play, enhance aesthetic appeal, take improved land out of use, etc. In these instances, Roundup Ready creeping bentgrass turf may no longer be managed to the same extent. To ensure undesired growth does not occur, superintendents or their employers will be responsible for devitalizing the unused Roundup Ready creeping bentgrass turf as well as monitoring and control of re-growth. Likewise, via a license agreement required for the golf course to initially purchase Roundup Ready creeping bentgrass seed, courses that are abandoned or put to some other non-golf use will be responsible for devitalizing the Roundup Ready creeping bentgrass turf, monitor the area for volunteers and devitalize any survivors. Similarly, sod farm fields may be removed from further sod production or used to produce sod of other varieties or species. In these cases, such field rotation will be handled in the manner described above.

E.6. Management of potential glyphosate-resistant weeds in Roundup Ready creeping bentgrass

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry #'s 1071-83-6), the active ingredient in the Roundup family of nonselective, foliar-applied, broad-spectrum, post-emergent herbicides (Baird, 1971; Malik et al., 1989), that is highly effective against the majority of annual and perennial grasses and broad-leaved weeds. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Steinrücken and Amrhein, 1980). The aromatic amino acid pathway is not present in mammalian metabolic pathways (Cole, 1985). Glyphosate has favorable environmental and safety characteristics, such as rapid soil binding (resistance to leaching) and biodegradation (which decreases persistence), as well as extremely low toxicity to mammals, birds and fish (Malik et al., 1989). Glyphosate is classified by the EPA as Category E (evidence of noncarcinogenicity for humans) (57 FR 8739).

Today, some 275 herbicide-resistant weed biotypes have been identified in various cropping systems in the U.S many of which are resistant to the triazine family of herbicides (Holt and Le Baron, 1990; Le Baron, 1991; Shaner, 1995). The development of resistance depends on a number of factors including chemical properties of the herbicide and its target site specificity, characteristics of the plant and agronomic practices. Historically, the onset of resistance to glyphosate has been far less than with other products. Based on current use data and the criteria described above, glyphosate is considered to be an herbicide with a low risk for weed resistance (Heap, 2001; Benbrook, 1991). After almost three decades of world wide use, confirmed resistance to labelled rates of glyphosate exists in very few species: biotypes of *Lolium rigidum* (annual ryegrass) in Australia, South Africa, and California; *Lolium multiflorum* (Italian ryegrass) in Chile; *Eleusine indica* (goosegrass) in Malaysia; and *Conyza canadensis* (marestail) in certain states of the eastern US.

The development of weed resistance to glyphosate is considered rare due to the following characteristics:

1. Most weeds and crops are inherently susceptible to glyphosate, and the long history of extensive use of glyphosate over the past 25 years has resulted in few instances of resistant weeds (Bradshaw *et al.*, 1997);

2. Glyphosate has many unique properties, such as its mode of action, chemical structure, limited metabolism in plants and lack of residual activity in soil, which makes the development of resistance less likely;

3. Selection for glyphosate resistance using whole plant and cell/tissue cculture techniques was unsuccessful, and is therefore, expected to occur rarely in nature under normal field conditions.

Nevertheless, a question has been raised about whether the introduction of crops tolerant to a specific herbicide, such as glyphosate, may lead to the occurrence of weeds resistant to that particular herbicide. This concern is based on the assumption that the use of the herbicide will increase significantly and that it will be used repeatedly in the same location.

However, it is important to recognize that weed resistance is an herbicide-related issue, not a crop-related issue. The use of a specific herbicide with an herbicide tolerant crop is no different than the use of a selective herbicide over a conventional crop from a weed resistance standpoint. While the incidence of weed resistance is often associated with repeated applications of an herbicide product, its development depends very much on the specific herbicide chemistry in question as well as the plant's ability to inactivate them. Some herbicide products are much more prone to develop herbicide resistance than others. Glyphosate has been used extensively for almost three decades with very few cases of resistance development largely due to many unique properties of glyphosate that makes the development of resistance unlikely. A brief summary of some of the factors affecting resistance and how they relate to glyphosate are described below.

E.6.a. Chemical properties of glyphosate

Mode of Action: Glyphosate shares the binding site on the EPSPS enzyme with the second substrate PEP and binds noncompetitively with respect to PEP. This means that after glyphosate binds to EPSPS, the enzyme is irreversibly deactivated. This also means that only a very few selective changes can occur within the enzyme to render it immune to glyphosate without interfering with normal function. Target site alteration is a common resistance mechanism among many herbicide classes, such as ALS-inhibitors and triazines. However, this mechanism is highly unlikely for glyphosate due to its high target site specificity such that modifications to the target site that could potentially confer resistance will almost always be lethal to the plant. This phenomenon was further illustrated in that selection for glyphosate resistance using whole plant and cell/tissue culture techniques was unsuccessful (OECD, 1999), and would, therefore, be expected to occur rarely in nature under normal field conditions.

Limited metabolism in plants: The lack of glyphosate metabolism or significantly slow glyphosate metabolism has been reported in several species and reviewed in various publications (Duke, 1988; Coupland, 1985). Because metabolism of the herbicide active moiety is one of the principle mechanisms for the development of herbicide resistance, it is unlikely that this mechanism would come into play with glyphosate.

Lack of soil residual activity: Glyphosate adsorption to soils occurs rapidly, usually within one hour (Franz et al., 1997). This binding makes glyphosate unavailable to plant roots and hence no impact to plants is observed from soil-bound glyphosate. The lack of any preemergence weed control from glyphosate is direct evidence of this phenomenon. Because glyphosate is not available to impact plants beyond the direct post-emergent application, the lack of sustained exposure or continuous pressure on weed populations reduces the likelihood of resistance development.

Nonetheless, as part of our product stewardship and customer service policy, Monsanto investigates cases of unsatisfactory weed control to determine the cause. Weed control failures following application of Roundup agricultural herbicides are most often the result of management and/or environmental issues and are rarely the result of herbicide resistance. However, for confirmed and suspected cases of resistance, Monsanto provides economical alternative control recommendations to the grower. To date, biotypes of only four weed species resistant to glyphosate have been identified (*Lolium rigidu, Eleusine indica, Lolium multiflorum* and *Conyza canadensis*). In all cases, Monsanto worked with local scientists to identify alternative control options that have been effective in managing the resistant biotype.

E.6.b. Stewardship implications

The key principles for effective stewardship of glyphosate use, including Roundup Ready crops, include: 1) basing recommendations on local needs and using the tools necessary to optimize weed control; 2) proper rate and timing of application; and 3) responding rapidly to instances of unsatisfactory weed control.

Monsanto has a system in place to investigate any reports of inadequate weed control in the field. Specially trained agronomists will consider a number of factors that could account for insufficient weed control, such as, application timing, application rates, growing conditions, weather, the particular weeds not controlled, etc. Almost all reports of unsatisfactory control are due to inappropriate application or environmental factors. If the problem occurs again in that field and it does not appear to be due to application or growing condition factors, plants are collected for further testing, and alternative weed control measures are provided.

Collected plants are scientifically analyzed through dose response and heritability studies in the greenhouse to determine if resistance has developed. If resistance is confirmed, the scientific and grower communities are notified as appropriate, and extensive analysis is initiated to elucidate the actual mechanism of resistance. Results of that research are publicly communicated at scientific meetings and in peer-reviewed scientific journals, and taken into consideration for continued stewardship recommendations.

Creeping bentgrass is grown as a perennial crop for seed for three years or more and is maintained as a turf on golf courses for many years. As stated above, the total amount of Roundup Ready creeping bentgrass seed needed to meet the demand of the golf course industry is expected to be grown on less than 3,000 acres. No more than two-dozen growers will be needed to produce this amount of seed. Consequently, the ability to regularly communicate and educate Roundup Ready creeping bentgrass seed producers on how to monitor for the signs of potential glyphosate resistance and report concerns to Monsanto and The Scotts Company will be greatly facilitated.

Sod producers, golf course superintendents and their staff will be well trained to monitor for the signs of glyphosate resistance, and Monsanto will aggressively monitor and

investigate any reports of inadequate weed control by Roundup from our Roundup Ready creeping bentgrass seed and sod producers or golf course customers. This has been the practice in every other Roundup Ready crop commercialized since 1996.

A typical golf course may have more than a dozen individuals responsible for cutting and maintaining the turf, in addition to the course superintendent. Consequently, every tee, green and fairway is inspected several times each week to ensure the exacting standards for playability and aesthetic quality demanded by course members and golfers are met. Such careful maintenance and inspection of the turf greatly facilitates the ability to immediately identify instances of unsatisfactory weed control for further investigation.

Furthermore, weed management recommendations for Roundup Ready creeping bentgrass will continue to be based on specific local needs and follow purposeful, basic weed management principles. Weed management practices shall be structured to include Roundup PRO herbicide, alone or in combination with other herbicides and/or cultural practices as required to deliver effective and economic weed control. For Roundup Ready creeping bentgrass grown on sod farms and golf courses, this may include the use of companion herbicides, such as one of a myriad of phenoxy or dicamba based pre-mixtures currently available in the market or newer chemistry such as isoxaben. These herbicides may be included as tank mixtures or as a sequential partner in combination with the use of Roundup PRO herbicide to form a state-of-the-art weed management program that will provide complete weed control and further contribute to effective, long term weed management.

E.7. Stewardship education and monitoring

The success of a stewardship plan is dependent upon the level of implementation, which in turn, dependent upon the level of understanding and commitment of the practitioner. The Scotts Company and Monsanto will educate Roundup Ready creeping bentgrass seed and sod producers and golf course superintendents on measures to limit the adventitious growth of Roundup Ready creeping bentgrass outside of their fields or courses and on steps to manage the potential for weed resistance to Roundup herbicides. The Scotts Company and Monsanto will also collaborate with the United States Golf Association (Greens Section), the Golf Course Superintendents Association of America, university extension agents and other organizations to develop educational materials and help ensure these stewardship goals are realized.

As stated above, specific stewardship requirements will be communicated with the Technology Use Guide prior to any Roundup Ready creeping bentgrass seed transactions. The Technology Use Guide will include details regarding the monitoring and options for control of feral populations of Roundup Ready creeping bentgrass or Roundup Ready creeping bentgrass hybrids, as well as monitoring for potential weed resistance to glyphosate. As an alternative, the golf course may develop their own stewardship best practices compatible with their own course conditions. The Scotts Company and/or Monsanto will review these stewardship plans. In either case, these guidelines will be communicated before a license to the technology is issued. These stewardship practices

will be included in the contractual agreement governing the use of Roundup Ready creeping bentgrass seed.

E.8. Summary

Roundup Ready creeping bentgrass has been demonstrated to exhibit the same agronomic and phenotypic characteristics as traditional creeping bentgrass, other than its tolerance to glyphosate. The Scotts Company and Monsanto have reviewed the potential for Roundup Ready creeping bentgrass gene flow to or from conventional creeping bentgrass varieties throughout the channel from seed production to the golf course. However, it is important to note the following.

- Creeping bentgrass is not considered a noxious weed other than grass seed production.

- There are mechanical means as well as several herbicides other than those based on glyphosate available to adequately control volunteer creeping bentgrass or Roundup Ready creeping bentgrass.

- The adventitious presence of the *cp4 epsps* gene in conventional creeping bentgrass or in species with which *A. stolonifera* can interbreed would pose no environmental risk since glyphosate tolerance confers no competitive advantage unless the plants are treated with glyphosate-containing herbicides.

The potential for the development of weed resistance to glyphosate-based herbicides on seed and sod production farms or golf courses was also evaluated. Stewardship practices have been developed to minimize inadvertent gene flow as well as to monitor and mitigate the potential development of glyphosate resistant weeds. Monsanto and The Scotts Company are committed to the successful implementation of these stewardship practices so the benefits of this product may be realized indefinitely.

F. Impact on non-target organisms

Observations taken from field testing of event ASR368 and progeny show that Roundup Ready creeping bentgrass exhibits no toxicity toward insects, birds or other species that may be present in creeping bentgrass seed or sod production fields or on golf courses (Section VI of this petition). EPSPS enzymes are present in plants and microorganisms, and are thus ubiquitous in nature and ordinarily present in food and feeds derived from plant and microbial sources. Therefore, the potential for harm to other organisms is considered to be no different than from other creeping bentgrass.

G. References

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VIII. Adverse Consequences of Introduction

Unfavorable grounds: NONE

Monsanto and The Scotts Company know of no unfavorable results or observations associated with event ASR368 that would result in adverse consequences of introduction. Therefore, on the basis of the substantial potential benefits to the grower, the environment and the consumer, Monsanto and The Scotts Company request a determination from APHIS that Roundup Ready creeping bentgrass event ASR368 and any progenies derived from crosses between lines of this event and other creeping bentgrass varieties no longer be considered regulated articles under regulations in 7 CFR part 340.

Appendix I

Agronomic, Environmental and Economic Benefits of Roundup-Ready® Creeping Bentgrass

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AGRONOMIC, ENVIRONMENTAL AND ECONOMIC BENEFITS OF ROUNDUP-READY® CREEPING BENTGRASS

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AGRONOMIC, ENVIRONMENTAL, AND ECONOMIC BENEFITS OF ROUNDUP READY® CREEPING BENTGRASS

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1. EXECUTIVE SUMMARY

The Monsanto and Scotts Companies have collaborated in the development of genetically modified turfgrasses that are tolerant to the otherwise nonselective herbicide, Roundup®, which contains glyphosate as the active ingredient. The first product slated for regulatory approval and market introduction is a creeping bentgrass cultivar that will be targeted for golf course fairway and putting green use.

1.1 Weed control benefits

Adoption of Roundup Ready® creeping bentgrass by golf courses can both simplify and improve the efficacy of weed control. A wide array of annual and perennial grasses, broadleaf, and sedge species can invade golf turf. These pests are currently controlled with variable success using a variety of herbicides and plant growth regulators (PGRs) that are applied throughout the growing season. These products number in the dozens and range from old phenoxy acid and arsenical compounds to more recent introductions such as ethofumesate and quinclorac, and PGRs such as paclobutrazol for annual bluegrass suppression.

1.2 Environmental and health benefits

An over-the-top treatment with a Roundup brand product will significantly reduce the need for many of these other herbicides. Exceptional need for these other herbicides may include the control of especially difficult weeds species, managing the unlikely development of a weed population resistant to glyphosate, or specific situations where the use of Roundup herbicide is inappropriate. In addition, certain fungicide and insecticide uses to manage annual bluegrass pests may be reduced when annual bluegrass is removed from mixed-species turfgrass stands.

Displacement of other pesticides with Roundup herbicide will potentially reduce exposure risks to human health and the environment. The toxicological, carcinogenicity, leaching, and runoff characteristics of glyphosate formulated as the professional-use product, Roundup Pro® herbicide, translate into a reduced potential risk for this technology versus other products commonly used for weed control on creeping bentgrass.

Roundup Pro herbicide has the fewest label warnings and least restrictive requirements for use, reflecting its overall lower risk of causing adverse effects to applicators and the environment. Glyphosate is classified as a Category E carcinogen, which is the lowest carcinogenicity risk assigned by the EPA. The vast majority of commonly used golf course pesticides have a carcinogenicity classification indicative of higher risk than glyphosate.

Glyphosate does not exceed the regulatory Level of Concern in worst-case risk analysis scenarios for fish, aquatic invertebrates, and aquatic plants. Hence the Roundup Pro label, unlike most other important golf course herbicides, does not include warnings of toxicity to fish and/or aquatic invertebrates and/or non-target plants.

1.3 Annual bluegrass management benefits

Particularly challenging grass weed problems in creeping bentgrass include annual and rough bluegrass because of their similar ecological adaptations, and fairway bermudagrass encroachment onto creeping bentgrass greens during heat stress periods. Overall, though, annual bluegrass is the most pervasive and troublesome weed in highly managed creeping bentgrass because it thrives and disperses viable seed under the same mowing, irrigation, and fertilization regimes as creeping bentgrass grown for fairway and putting green uses. In fact, annual bluegrass alone is considered a viable playing surface in a very limited number of golf course environments because of its density, vigor, and tolerance to shade and close mowing.

In most environments, annual bluegrass suffers from a variety of cold hardiness, heat tolerance, and disease and insect pest susceptibility problems that creeping bentgrass is not susceptible to and which limit its utility as a perennial turfgrass. Most important, annual bluegrass frequently fails under the heat and drought stress conditions of midsummer because it is best adapted to cool, moist conditions. The result of its aggressive cool-season colonization and marginal warm-season survivability is that golf course superintendents invest a great deal of labor, chemistry, water, and intellectual energy into managing annual bluegrass in mixed stands with creeping bentgrass – either in an attempt to eliminate it or to encourage its survival in situations where control strategies have proven futile.

Just the potential elimination of annual bluegrass with Roundup herbicide from Roundup Ready creeping bentgrass fairways offers numerous associated agronomic and environmental advantages:

- Potential replacement of more than 125,000 lb (active ingredient, or ai) of fumigants annually - including 63,000 lb ai of methyl bromide - with approximately 1,500 lb ai of Roundup for turfgrass establishment
- Potential elimination of more than 300,000 lb ai of annual fungicide treatments for anthracnose and summer patch diseases
- Potential elimination of nearly 16,000 lb of annual insecticide treatments in the northeast US for annual bluegrass weevil
- Potential elimination of more than 17,000 lb ai of annual PGR treatments for annual bluegrass suppression and bentgrass conversion
- Improved creeping bentgrass performance as a result of single-species management focus

The varying degree to which annual bluegrass management impacts individual golf course operations makes assessing the overall benefits of Roundup Ready creeping bentgrass difficult. In certain cases, drastically different approaches to managing pure creeping bentgrass may be

adopted, such as the complete elimination of fumigants at establishment and shifts in cultural programs. In other cases, the availability of the technology may prompt conversions from other species with their own management challenges. This benefits assessment only addresses the most obvious changes in management practice, so it is likely to be conservative in scope.

1.4 Additional turfgrass management benefits

General changes to weed control and turfgrass management from the adoption of Roundup Ready creeping bentgrass can produce these additional benefits:

- Potential replacement of more than 75,000 lb ai of annual herbicide treatments with a similar amount of Roundup herbicide for control of grass and broadleaf weeds
- Reduced creeping bentgrass injury from the use of marginally selective herbicides
- Labor reductions from reduced pesticide applications and annual bluegrass cultural management
- Increased golfer (customer) satisfaction from improved playability and performance of fairway surfaces

1.5 Regulatory approval and product stewardship

In support of regulatory clearance prior to commercialization, the Scotts Company and Monsanto are developing a separate technical stewardship strategy for selective Roundup herbicide use in golf course operations. Issues being addressed include risks to non-target vegetation and weed management strategies for various turfgrass management scenarios. Management of potential weed resistance to Roundup herbicide is addressed in separate documents contained in other crop submissions. Weed resistance to Roundup herbicide is a rare occurrence globally after 20 years of use and is not expected to warrant a special management program.

In summary, the introduction of Roundup Ready creeping bentgrass to the golf course market offers opportunities to (1) simplify turfgrass management, (2) improve financial performance through reduced agronomic and labor inputs, and (3) improve the competitive positioning of adoptive golf courses for attracting discriminating golf clients. The use of a Roundup brand herbicide product, with glyphosate's low toxicity and favorable environmental profile, provides significant improvements over existing management tools, as does the complete elimination of certain fungicide, insecticide, and PGR applications.

2 INTRODUCTION

This paper identifies and discusses the various benefits and pertinent management issues associated with the introduction of creeping bentgrass (*Agrostis stolonifera* L.) that is tolerant to glyphosate, the active ingredient in Roundup branded herbicides (henceforth "Roundup herbicide") a nonselective herbicide, to the golf course turfgrass market.

2.1 Creeping bentgrass management on golf courses

Creeping bentgrass is widely planted for golf course putting green, tee, and fairway turf in the cooler climates throughout the United States, and exclusively for greens in the south where specialized root zone management systems allow survival of creeping bentgrass under summer heat conditions. A 1999 golf course survey (Doane Marketing Research, Inc.) estimated that 11,800 US golf courses manage a collective 24,570 acres of creeping bentgrass greens and 6,950 tees, or an average of 2.1 and 2.0 acres per course, respectively.

National Golf Foundation (2001) data indicate that about 16,140 golf courses were in operation at the time of the Doane survey. These figures suggest that creeping bentgrass is grown on approximately 70% of US golf greens and 20% of tees. The Doane (1999) survey also estimated that fairways accounted for 52,650 acres of creeping bentgrass over 2,300 golf courses, an average of 23 acres per course. Using the National Golf Foundation data, this represents about 15% of US fairways.

The National Golf Foundation (2001) reports that about 17,100 golf courses are in operation today, a 6% increase over 1999. Applying this growth factor to creeping bentgrass suggests a current US inventory of 26,000 putting green acres on 12,500 golf courses, 7,360 tees on 3,530 courses and of 55,800 fairway acres on 2,440 golf courses.

Nationwide acreage of fairway creeping bentgrass is slightly more than twice that of greens. On golf courses where both greens and fairways are planted to creeping bentgrass, fairway acreage is approximately five times that of greens and tees. Overall, though, fairway, tee and putting green acreage accounts for only 15-20 percent of a typical golf course property that includes less managed roughs and natural landscapes, a practice range, maintenance facility, clubhouse and parking lots, and tennis and swimming facilities (Beard, 1982). These areas do not typically support the agronomic needs of creeping bentgrass.

Management of putting greens is considerably more intensive than for fairways with regards to mowing height and frequency, irrigation, nitrogen use, disease control, and thatch management. For example, golf courses spent similar amounts in 1999 to control summer patch disease on annual bluegrass in creeping bentgrass greens and fairways ($2.52 million and $2.96 million, respectively), even though greens acreage in the survey was less than half that of fairways (Monsanto, internal market research). Nitrogen inputs for greens range from two to three times per unit area as for fairways (Beard, 1982). Golfers' performance demands for the putting green surface are strict, so golf course superintendents are slower to adopt new technologies there than on fairways (Zontek, 2001). However, the increased level of management inputs to greens

suggests that benefits described in this paper for fairways will be even greater per unit area on greens.

Few stands of creeping bentgrass maintained under golf course conditions remain pure because they are readily invaded by naturally occurring populations of annual bluegrass (*Poa annua* L.) within a few years (Beard, 1982). Annual bluegrass can out-compete creeping bentgrass under fairway and putting green management conditions during cool, moist periods or in shaded environs. Annual bluegrass produces large amounts of seed, which can germinate continuously throughout the year, and seedlings are quite aggressive. The result is a patchwork of creeping bentgrass and annual bluegrass that select between micro-environmental differences in mowing height, shade, drainage, and soil pH (Watschke, 1995).

The typically patchy species mixture of creeping bentgrass and annual bluegrass complicates turfgrass management because creeping bentgrass is more competitive than annual bluegrass under hot, summer conditions while annual bluegrass performs best in the cool, moist conditions of early spring and late fall (Christians, 2000). Annual bluegrass can decline or die from midsummer heat stress, leaving colonized areas ungrassed or bare and compromising course playability at a time of year when it is difficult to encourage creeping bentgrass to fill in those areas. As a result, golf course superintendents invest significant time and money into the cultural and chemical management of annual bluegrass in creeping bentgrass.

2.2 Roundup herbicide use and risk considerations

Roundup herbicide is widely used in agriculture for no-till cropping and in landscape management for directed applications to control weeds in turfgrass, ornamental plantings, and nonvegetated areas such as parking lots. The active ingredient of Roundup herbicide is glyphosate (N-phosphonomethyl-glycine), registered with the EPA by Monsanto in 1974 (Monsanto, 2000).

2.1.1 Comparison of Roundup herbicide risks versus other herbicides

Glyphosate is considered to have a more favorable human health and environmental risk profile than many other currently available golf course pesticides. To support this conclusion, an abbreviated comparative analysis of risk characteristics for some important labeled products versus glyphosate (formulated as the professional-use product, Roundup Pro® herbicide) is presented here. Comparative summary tables can be found in Appendix A, and include information obtained from US EPA publications in the Federal Register, Pesticide Fact Sheets, Reregistration Eligibility Decision documents and the EPA Ecotoxicity One-Liner Data Base. In addition, this assessment utilizes information in the USDA Pesticide Properties database, Product Labels and in published references such as the Weed Science Society's Herbicide Handbook.

2.1.1.1 Comparison of herbicide use hazards

Various hazard parameters, warning statements, use requirements and restrictions for some commercially important herbicide products are compared with those of glyphosate and Roundup Pro herbicide in Appendix A, Tables 1 and 2. This comparison reveals that Roundup Pro herbicide has the fewest label warnings and least restrictive requirements for use, reflecting its overall lower risk of causing adverse effects to applicators and the environment. Glyphosate has relatively favorable acute chronic, carcinogenic, reproductive, and developmental toxicity profiles. Glyphosate also binds tightly to soil and degrades readily, and therefore will not leach and is not likely to reach surface water.

Roundup Pro herbicide carries the CAUTION! signal word on its label and has the least restrictive Personal Protective Equipment (PPE) requirements for applicators and handlers, compared to nearly all other alternative products in this analysis. One product, Millenium Ultra, carries the DANGER! signal word because it carries more acute exposure risk for applicators due to the acute toxicity findings requiring this signal word. Similarly, three other products in this comparison carry the WARNING! signal word, indicative of a moderate exposure risk.

2.1.1.2 Roundup herbicide risks to human health

Of the herbicide active ingredients listed in Appendix A for which the Carcinogen Classification was available, only glyphosate is classified in Category E, which is the lowest carcinogenicity risk assigned by the EPA. In fact, the vast majority of commonly used golf course pesticides have a carcinogenicity classification indicative of higher risk than glyphosate. Of the hundreds of pesticide active ingredients registered by the EPA, only twenty-four carry the Group E classification and only six of these are approved for golf course applications. None of these six products are routinely used on golf courses, only for highly specialized applications.

The EPA determined in recently completed aggregate exposure assessments for dicamba, 2,4-D, and clopyralid under the Food Quality Protection Act (FQPA) that it was necessary to apply extra FQPA Uncertainty Factors in considering the protection of infants and children. Aggregate exposure assessments under the FQPA do not account for occupational, non-crop, non-residential uses on turf such as golf course applications, but instead evaluate risks from exposure to pesticide residues through food, water and residential uses. The point of referencing these otherwise unrelated decisions is that the requirement of an uncertainty factor is typically indicative of developmental or reproductive effects seen in available toxicological studies. No such effects have been found in multiple studies conducted on glyphosate.

2.1.1.3 Roundup herbicide risks to non-target organisms

Hazard information regarding non-target organisms for formulated end-use products is not readily available, so this comparison is based on active ingredients. Related label warnings are also examined.

Estimated exposure and hazard information for non-target species is summarized in Appendix A, Tables 3, 4 and 5, along with quantitative comparisons of Risk Quotients. Risk Quotients

(RQs) compare estimates of environmental exposure relative to a toxicological endpoint, in this case a fish LC_{50} or plant/invertebrate EC_{50}. XC_{50}s are exposure doses or concentrations that are lethal to 50% of a test population and are a standard expression of relative pesticide toxicity. Environmental exposure was estimated for all products using standard EPA assumptions of 5% drift and 5% runoff of applied pesticides into adjacent water and maximum single application rates for turf from product labels. Risk Quotients of 0.5 (50% of the XC_{50}) and higher are considered Levels of Concern in ecological risk assessments by the EPA.

In this analysis, bensulide, oxadiazon, dithiopyr, pendimethalin, and prodiamine each exceed the Level of Concern in at least one worst-case risk scenario for fish, aquatic invertebrates, and aquatic plants. Based on the range of RQs, glyphosate products are considered to represent a general reduction in risk to aquatic species and non-target plants compared to the alternative products. Unlike Roundup Pro – which has no warnings relative to non-target, aquatic species - the labels for products containing bensulide, oxadiazon, dithiopyr, pendimethalin, 2,4-D, clopyralid, prodiamine and fenoxaprop include warnings of toxicity to fish and/or aquatic invertebrates and/or non-target plants.

2.1.1.4 Roundup herbicide risks to water quality

Appendix A, Table 6 presents a comparison of the water contamination risk potential for glyphosate and the alternative herbicides. Ground Water Ubiquity Scores (GUS) were calculated as an indicator of relative leaching potential (Gustafson, 1989). The GUS index ranges as shown in Table 7 can be used to group chemicals into high, medium, or low potential for leaching and, indirectly, for surface runoff. Glyphosate has a low potential for leaching or runoff. Four of those products with available information - dicamba, 2,4-D, clopyralid and paclobutrazol - are shown to have a medium or high risk of leaching based on GUS calculations. Glyphosate would offer a potential reduction in risk for ground water and surface water contamination compared to these alternatives.

2.3 Glyphosate mode of action and tolerance in plants

Glyphosate's herbicidal activity results from interference with normal plant metabolism by inhibiting a critical enzyme, 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS), which is involved in the biosynthesis of aromatic amino acids, vitamins, and many secondary metabolites. Inhibition of aromatic amino acid biosynthesis disrupts protein synthesis and results in the plant's death (Organization for Economic Cooperation and Development, 1999). EPSPS is specific to plants and some microorganisms but does not occur in higher animals.

Roundup Ready plants were developed through the introduction of a naturally glyphosate tolerant form of EPSPS from Agrobacterium into creeping bentgrass. Such genetically modified plants, differing only in the introduction of this gene and encoded EPSPS protein, are able to tolerate glyphosate treatment at levels to control key weed pests.

A number of crop plant species have been transformed using recombinant DNA techniques to express glyphosate tolerance (Organization for Economic Cooperation and Development, 1999). Crops already deregulated by the USDA for commercial production include soybean,

cotton, corn, sugar beet, and canola (USDA, 2000; USDA 2001). Initial US commercialization of Roundup Ready crops began in 1996 with soybeans, followed by cotton in 1997 and by corn and canola in 1999. Estimates of planted acres for 2000 indicate that these four Roundup Ready crops accounted for tens of millions of US production acres and hundreds of millions of acres worldwide (ISAAA, 2000). The multiple years and rapid adoption of Roundup Ready crop production translate into a substantial scientific and grower knowledge base and have demonstrated the favorable toxicological, environmental and economic properties of this technology.

Monsanto and The Scotts Company have applied these recombinant DNA techniques to impart glyphosate tolerance to creeping bentgrass, thus allowing direct applications of Roundup herbicide. Introduction of a commercial cultivar to the marketplace is expected in the near future, following appropriate regulatory approval. The rest of this paper discusses the golf course and associated management impacts of that proposed market introduction.

3. WEED MANAGEMENT AND HERBICIDE USE

3.1 General weed control impacts

The most direct impact of planting Roundup Ready creeping bentgrass on golf courses will be the ability to spray the herbicide directly over the turf sward and to selectively control weeds, including other grasses. This capability has the potential to drastically simplify, improve efficacy, and reduce the cost of weed control programs by allowing Roundup herbicide to replace the routine application of the wide variety of grass and broadleaf herbicides currently in use. Most other herbicides do not exhibit the same favorable environmental and human health profile of Roundup herbicide, so reducing their use can provide the significant benefit of reducing risks to human health, wildlife, and the environment. Moreover, Roundup will not injure Roundup Ready creeping bentgrass under the recommended conditions of use but is completely effective against most turfgrass weeds, while many other available herbicides can injure creeping bentgrass and/or are not completely efficacious for the full spectrum of weeds. Finally, Roundup's post-emergence use pattern allows directed-application to localized weed infestations in lieu of broadcast applications of pre-emergence products. Thus, the introduction of Roundup herbicide to the turfgrass weed management program can improve weed control efficacy, reduce potential turfgrass injury, and reduce total herbicide use; benefiting both the golf club and the golfer via improved playability and more effective and economical turfgrass management.

Current weed control strategies in turfgrass management programs vary widely according to the weed species present on each golf course, which can be effected by geographic region, the age of the stand, weather anomalies, and a host of other factors. General classifications of turfgrass weeds include broadleaf, grass, and sedge plant families; and annual, biennial, and perennial life cycles (Watschke, 1995). These classifications generally suggest herbicide strategies and products for weed control such as pre-emergence products for annual weeds versus post-emergence products for perennials and/or annuals, or grass-specific versus broadleaf specific products. Table 1 categorizes some weeds common to creeping bentgrass fairways. The list is by no means comprehensive for all golf courses in all bentgrass-growing regions.

Table 1. Common creeping bentgrass weed species and classifications.

WEED CLASSIFICATION	COMMON SPECIES
(Summer) Annual Grass	Crabgrass species, goosegrass, foxtail sp., panicum sp., barnyardgrass
Winter Annual Grass	Annual bluegrass, annual ryegrass
Perennial Grass	Quackgrass, nimblewill, *Allium* sp., rough bluegrass, perennial ryegrass, bermudagrass, zoysiagrass, dallisgrass
(Summer) Annual Broadleaf	Black medic, knotweed, spurge, purslane
Winter Annual Broadleaf	Corn speedwell, yellow rocket
Perennial Broadleaf	Creeping speedwell, mouse-ear chickweed, dandelion, ground ivy, heal-all, plantain sp., white clover
Sedge	Yellow nutsedge

Sources: Watschke, 1995; Beard, 1982

3.2 Herbicide use impacts

Numerous herbicide products and timings are necessary to effectively manage this array of weed species and types. Table 2 summarizes herbicide types, products, and weed targets that are applicable to creeping bentgrass fairways.

Table 3 outlines a herbicide schedule that is typical for weed control in golf course fairways in the northern United States. Not every application will necessarily be made every year depending on weed pressure, and some applications are alternatives for others. In the first instance, post-emergence grass treatment may not be necessary if a pre-emergence treatment is effective. In the latter scenario, a post-emergence grass treatment can be an alternative strategy to pre-emergence herbicides if weed pressure is known to be light or if pre-emergence treatments present environmental or turfgrass injury risks. A standard herbicide program is likely to consist of spring treatments with pre-emergence grass and post-emergence broadleaf materials, followed by spot treatments for weed escapes. This herbicide discussion does NOT account for post-emergence plant growth regulator applications to manage annual bluegrass, which is addressed in a later section of the paper.

Table 2. Herbicide types, products, and target weeds common to creeping bentgrass fairways.

HERBICIDE TYPES AND PRODUCTS	WEEDS CONTROLLED
Pre-emergence grass: Pendimethalin, prodiamine, dithiopyr, benefin, bensulide, siduron, oxadiazon, ethofumesate	Summer and winter annual grasses
Post-emergence grass: Fenoxaprop, dithiopyr, ethofumesate methyl arsonate, some marginal activity from preemergence grass herbicides	Summer and winter annual grasses and some perennial grasses
Pre-emergence broadleaf: Isoxaben, some marginal activity with pre-emergence grass herbicides	Winter annual broadleaves
Post-emergence broadleaf: Phenoxy acids, dicamba, triclopyr; clopyralid	All broadleaves (perennials require repeat applications)
Pre/Post-emergence combinations: Commercial and tank mixed combinations of listed products	
Other: Bentazon, halosulfuron	Yellow nutsedge
Nonselective: Glyphosate, glufosinate, diquat, paraquat	All species

Sources: Landschoot, 1994; C&P Press, 1998; Dernoeden, 2000

Table 3. Typical herbicide treatments and timings for turfgrass in the northern United States.

TIMING	HERBICIDES	TARGET WEEDS
April-May	Pre-emergence grass	Summer annual grasses
May-June	Post-emergence broadleaf	Annual and perennial broadleaves
June-July	Post-emergence grass	Summer annual grasses
July-September	Post-emergence grass Post-emergence broadleaf Non-selectives	Directed spot treatments for weed escapes
September-October	Pre-emergence grass ---------- Pre-emergence broadleaf Post-emergence broadleaf	Winter annual grasses ---------- Biennial broadleaves

Source: Watschke, 2001; Dernoeden, 2000

This complex program of treatments can potentially be replaced by three strategic applications of Roundup herbicide, which are described in Table 4. Cost impacts are evaluated in a later section.

Table 4. Proposed weed control program using Roundup herbicide in place of traditional turfgrass herbicides

TIMING	TARGET WEEDS
April-May	Winter annuals
June-July	Broadleaves and annual grasses
Aug-Sept	Goosegrass and winter annuals

Projected weed control benefits of the Roundup herbicide program include

- Reduction of pre-emergence herbicide use
- Reduction in overall pounds of herbicides applied
- Potential reduction in the number of seasonal herbicide applications
- Control of perennial grass weed species, many for which there are no selective controls

Pre-emergence herbicides can interfere with over-seeding programs, which are critical to maintaining stand density for high quality playing surfaces. Also, some pre-emergence herbicides can injure or delay growth of creeping bentgrass roots, thereby reducing heat and drought tolerance (Dernoeden, 2000).

Moreover, their pre-emergence function is typically characterized by a longer half-life than glyphosate, thus they provide residual control throughout the seasonal germination period. Longer half-lives mean pre-emergence herbicides are generally more persistent in the environment. The suggested soil half-life value for glyphosate from the USDA Pesticide Properties Database is 7 days, with a range of 2-174 days. Conversely, the average half-life of the nine pre-emergence grass and broadleaf herbicides listed in Table 2 is 115 days, with a range of 30 to 400 days. (USDA, 2000).

It is unlikely that Roundup herbicide will completely replace existing herbicide usage in Roundup Ready creeping bentgrass. The control of specific weeds with regard to efficacy, application timing, and application method may benefit from the use of other herbicides. Scotts and Monsanto are actively pursuing technical strategies to evaluate and address these issues.

3.3 Perennial grass weeds

An important cultural benefit of Roundup herbicide beyond potentially reducing most applications of other herbicides is the control of perennial grasses such as rough bluegrass, perennial ryegrass, bermudagrass, quackgrass, and dallisgrass. There are currently no selective chemical controls for perennial grass weeds in creeping bentgrass. Aside from annual bluegrass management, which is addressed in detail in a subsequent section, perennial grasses are of the most difficult weed control challenges in fine turf. Two scenarios are of particular concern to the industry and are discussed here.

3.3.1 *Poa trivialis*

Poa trivialis L., commonly called rough or rough-stalked bluegrass, is a perennial grass weed that is both naturalized in northern areas of the United States (Hurley, 1997) and also is recognized as a major contaminant of grass seed grown in the Pacific Northwest (Levy, 1998). Since the species is imported, naturalized populations are generally remnants of previous contaminated plantings. Current seed contamination results from a number of factors including production for a southern over-seeding market that creates sanitation challenges to commercial handling systems, as well as naturalized (weed) populations in seed growing areas (Liskey, 1999).

Once established, *Poa trivialis* spreads by stolons and can form large, contiguous patches that displace desirable cool-season grasses such as creeping bentgrass. *Poa trivialis* patches tend to be unsightly and can affect the quality of golf play due to their distinctly different coloration and texture. Moreover, *Poa trivialis* exhibits poor heat and drought tolerance (much like *P. annua*), which leads to its decline or dormancy in the summer. For this reason, superintendents currently go to great lengths to exclude the weed from fairways and greens including hand removal, spot treating with nonselective herbicides, and even complete renovation (Zontek, 2001).

Poa trivialis is not as prolific a seed producer as annual bluegrass, so it has less of a seed bank in native soils. However, its spreading habit makes it a formidable colonizer in its own right. In addition, its persistence as a seed contaminant means that it is introduced routinely to golf courses, which then must embark on a program to rogue it out of the new seedings before it begins to compete and spread. The introduction of Roundup Ready creeping bentgrass will provide an opportunity to eliminate this persistent and troublesome pest with Roundup and with far less labor than is currently required.

3.3.2 Other turf-type perennial grasses

Another scenario that presents difficult perennial weed management challenges is the conversion between turfgrass species on golf holes or entire golf courses. The practice has been particularly prevalent in the middle latitudes of the United States, commonly referred to as the transition zone, where cool-season and warm-season grass growing regions overlap and conditions are frequently not optimum for either system.

It has been common for transition zone golf courses to plant or convert several turfgrass species including Kentucky bluegrass, perennial ryegrass, bermudagrass, zoysiagrass, and/or creeping bentgrass to identify the best candidate for a particular microclimate. To facilitate conversion, existing stands are generally killed with a nonselective herbicide prior to planting the new species. In cases where herbicide performance is less than perfect, escapes from the old population often survive treatment and re-establish as a competitor to the new turfgrass of choice. The situation can be particularly annoying to managers and golfers when the competing species are noticeably different in texture, color, or growth habit. Bermudagrass and zoysiagrass escapes in creeping bentgrass conversions are commonly seen throughout the transition zone.

In a similar fashion, these warm-season grasses – particularly bermudagrass - can invade creeping bentgrass greens when they are grown on adjacent fairways and greens surrounds. In either case the lack of effective controls makes their elimination quite problematic. The availability of Roundup Ready creeping bentgrass will allow the selective removal of competitive perennial grasses from creeping bentgrass greens and fairways with repeated applications of Roundup.

4. ANNUAL BLUEGRASS MANAGEMENT CONSIDERATIONS

4.1 The annual bluegrass paradox

Annual bluegrass represents a dilemma for many golf course superintendents: In cool, humid periods it out competes creeping bentgrass and can provide a superior playing surface, but then it performs poorly or even fails completely under the heat stress conditions of mid-summer to which creeping bentgrass is quite tolerant. Winter injury, particularly damage from ice encasement, is common to annual bluegrass as well (Watkins, 2001; Christians, 1996; Perrault, 1998; Tegtmeier, 1997). While technically a pest, the competitiveness of annual bluegrass under conditions that are adverse to creeping bentgrass can be desirable to putting surfaces throughout the year and particularly for early spring and late autumn golfers (Oatis, 2001). These same customers are not understanding, though, when annual bluegrass declines during the summer golfing season.

Another important limitation of annual bluegrass is its aggressive production of seedheads and viable seed. The seed disseminates to constantly replenish the seed bank, perpetuating the problem for managers seeking to eliminate annual bluegrass. Seedheads are not only unsightly, but physically disrupt putting by producing an uneven surface that interferes with ball roll. As a consequence, golf course superintendents use plant growth regulators such as mefluidide (Embark T&O) to suppress seedheads and collect clippings to avoid returning seed to the soil. Embark T&O, however, performs erratically and can be phytotoxic to creeping bentgrass (Dernoeden, 2001).

Roundup Ready creeping bentgrass offers a unique mechanism to limit the colonization of creeping bentgrass stands by annual bluegrass. This development provides an opportunity to manage this pest like a true weed instead of a troublesome colonist that must be tolerated. The result will be simplified management of a single turfgrass species, lower management input costs, improved agronomic bentgrass performance, and improved bentgrass playability.

4.2 Annual bluegrass management choices

Many golf course superintendents currently feel that annual bluegrass elimination is a reasonable goal for fairways, but not for greens (Watkins, 2001; Oatis, 2001). Even though creeping bentgrass tolerates the extremes of summer and winter weather better than annual bluegrass, annual bluegrass can still provide a high quality putting surface. It is more tolerant than creeping bentgrass of shade, excess soil moisture, and soil compaction, and grows more aggressively in cold (non-freezing) weather. The combined stresses of traffic, greens construction and root mix imperfections, and local environmental conditions can result in temporary, localized decline of creeping bentgrass even under the best management regimes (Oatis, 2001). In those cases, annual bluegrass often fills in and provides a form of putting quality "insurance." Given the intolerance of golfers and, consequently, course management for inconsistent or poor putting conditions, most superintendents choose to accept some annual bluegrass colonization on putting greens. The intense management practiced on the relatively small acreage represented by greens makes this choice possible and even practical in most instances. Without annual bluegrass colonization to fill voids in the creeping bentgrass on greens, many superintendents express concern for their job security (Watkins, 2001).

This widespread resignation to annual bluegrass infestation of creeping bentgrass greens is clearly a quality tradeoff that complicates turfgrass management and fails to optimize putting conditions for either species. While each species has biological limitations in a mono-stand that affect their individual utility as a putting surface, pure annual bluegrass is simply not an option in most regions because it is not a true perennial and declines after seed set and in the peak of summer. Pure creeping bentgrass greens are a better quality and more reliable playing surface than mixed stands where a playable condition can be satisfactorily maintained throughout the golfing season.

Unlike putting greens, most golf course superintendents prefer to manage annual bluegrass *out* of creeping bentgrass fairways using pre-emergence herbicides, plant growth regulators, and aggressive cultural programs (Christians, 2000; Dernoeden, 2000; Watkins, 2001). This choice is practical because of a higher tolerance on fairways than greens for quality variations after chemical treatments to annual bluegrass, the increased competitiveness against annual bluegrass of fairway height (0.5+ in.) creeping bentgrass compared to greens mowing heights (<0.16 in.), and the overall lower management effort invested in fairways than greens. Regardless of these eradication efforts, though, few superintendents are able to achieve better than 85% bentgrass purity because annual bluegrass is such an aggressive competitor in the biological niche created by putting green and fairway management (Watschke, 2001).

4.2.1 Management opportunities with Roundup Ready creeping bentgrass

Roundup Ready creeping bentgrass offers an opportunity to pursue this industry goal of adequately maintaining pure creeping bentgrass on both fairways and putting greens. Routine elimination of annual bluegrass with Roundup herbicide means that superintendents can initially improve creeping bentgrass quality throughout the year by optimizing management inputs according to the season. In those isolated locations where creeping bentgrass still cannot satisfy golf performance requirements, superintendents may choose to forego the Roundup herbicide

treatment and maintain the annual bluegrass. This flexibility is a critical benefit of the Roundup Ready creeping bentgrass program, because it allows superintendents the ability to maximize turfgrass quality in all growing environments.

Future breeding efforts will be directed at the development of Roundup Ready creeping bentgrass cultivars with improved performance under cool, shady, and moist conditions without persistent competition from annual bluegrass.

4.3 Specific impacts of annual bluegrass control with Roundup herbicide

The following comments are made primarily with fairway applications in mind because fairways represent the largest creeping bentgrass acreage on most golf courses. Direct cost comparisons are presented in a later section.

4.2.2 Simplified creeping bentgrass management

Managing mixed stands of creeping bentgrass and annual bluegrass is a complex process because the two species benefit from many of the same routine cultural practices such as close mowing, abundant water and nutrients, and aggressive cultivation. The result is that each is able to prevail in specific micro-environments throughout the golf course based on seasonal temperature (as affected by light, elevation, and aspect), moisture (as affected by soil type, drainage, compaction, relief, and irrigation efficiency), nutrient balance (as affected by application efficiency, soil type, drainage, and nitrogen conversion) and pH (as affected by soil type, application efficiency, drainage). Most attempts to completely eliminate annual bluegrass with chemicals from a mixed stand have resulted in unacceptable injury to the creeping bentgrass. Instead, golf course superintendents typically supplement chemical applications with management practices that subtly tip the ecological balance of water, temperature, pH, and plant vigor toward optimum creeping bentgrass conditions.

Table 5 compares current management strategies and their limitations for mixed stands with potential Roundup Ready creeping bentgrass (RRCB) practices and advantages. Current fairway management practices discourage annual bluegrass, sometimes at the expense of creeping bentgrass needs for water, fertilizer, or aeration. Exceptions occur during periods of tournament and similar showcase preparations, when annual bluegrass condition will be enhanced until playability demands can be reduced.

Most of the simplified or optimized management benefits in Table 5 are performance-related and financially intangible. Exceptions are pesticide costs and labor reduction benefits from reduced hand irrigation and fewer pesticide applications. Late season cultivation and overseeding add expenses that are offset by improved turfgrass condition and delayed renovation cost. Economic assessments of these effects will be addressed in subsequent sections.

Table 5. Comparison of mixed-stand versus Roundup Ready creeping bentgrass management practices and implications

SOIL REACTION	
Current Practice	Maintain pH 5.5 to 6.0, to discourage annual bluegrass
Current Limitation	Creeping bentgrass optimum pH range extends to 6.5, which overlaps with annual bluegrass range of 6.0 to 7.0
RRCB Option	Maintain optimum bentgrass pH range of 5.5 to 6.5
RRCB Advantage	Improve overall soil environment for creeping bentgrass
SPRING AND FALL FERTILIZATION	
Current Practice	Minimize fertilization in the early spring and late autumn to avoid stimulation of annual bluegrass
Current Limitation	Restricted fertilization period reduces bentgrass vigor
RRCB Option	Extended fertilization season
RRCB Advantage	Extended bentgrass growth season
PREEMERGENCE HERBICIDES	
Current Practice	Apply preemergence herbicides prior to annual bluegrass germination
Current Limitation	Annual bluegrass preemergence timings preclude early season and late summer bentgrass overseeding opportunities and increase risk of phytotoxicity to creeping bentgrass
RRCB Option	Reduce or eliminate preemergence grass herbicides; directed sprays in lieu of broadcast applications
RRCB Advantage	Minimize bentgrass root stress and phytotoxicity; allow spring/fall overseeding; reduced pesticide use
PLANT GROWTH REGULATORS	
Current Practice	Apply paclobutrazol to reduce annual bluegrass vigor in fairways
Current Limitation	Narrow application timing window
RRCB Option	Eliminate paclobutrazol
RRCB Advantage	Pure stand management
SUMMER FERTILIZATION	
Current Practice	Fertilize creeping bentgrass only in summer with frequent, low-nitrogen applications of foliar formulations when annual bluegrass is under heat stress
Current Limitation	Aggressive summer fertilization can increase bentgrass disease risk
RRCB Option	Optimize seasonal fertilization schedule
RRCB Advantage	Reduce disease risk, improve early and late season creeping bentgrass condition

Table 5 (cont'd). Comparison of mixed-stand versus Roundup Ready creeping bentgrass management practices and implications

IRRIGATION	
Current Practice	Irrigate deeply and then allow bentgrass to approach wilt conditions between irrigations to reduce annual bluegrass competition
Current Limitation	Heavy traffic during moisture stress periods (for annual bluegrass management) can reduce bentgrass recuperation
RRCB Option	Optimize irrigation for creeping bentgrass performance
RRCB Advantage	Maintain soil moisture for better recuperation from play stress
DISEASE AND INSECT MANAGEMENT	
Current Practice	Treat summer patch, bacterial wilt, and anthracnose diseases and annual bluegrass weevil as needed
Current Limitation	Not required or reduced need (anthracnose) for pure creeping bentgrass
RRCB Option	Reduce or eliminate pesticide treatments
RRCB Advantage	Reduce human and environmental exposure; eliminate cost
SYRINGING/HAND WATERING	
Current Practice	Prescription watering during midday heat stress to avoid unrecoverable annual bluegrass decline
Current Limitation	Labor, frequency, effectiveness, lack of annual bluegrass recovery
RRCB Option	Hand water bentgrass only under severe heat stress conditions
RRCB Advantage	Less need, severe heat effects are recoverable
MOWING HEIGHT	
Current Practice	Raise mowing height whenever possible to increase bentgrass competitiveness over annual bluegrass
Current Limitation	Golfers prefer lower mowing height for performance, which favors annual bluegrass in competitive stands
RRCB Option	Lower mowing height for golfers
RRCB Advantage	Playability, business competitiveness
AERATION/OVERSEEDING	
Current Practice	Avoid aeration and overseeding in growing season to minimize annual bluegrass re-colonization from seed bank
Current Limitation	Highly beneficial agronomic practice is minimized
RRCB Option	Perform spring and/or late summer cultivation and overseeding operations
RRCB Advantage	Maintain stand density, better thatch management

Sources: Beard, 1982; Dernoeden, 1995; Dernoeden, 2001; Watschke, 1995; Watschke, 2001; Vittum, 2001

4.2.3 Reduced management inputs

Direct cost effects of Roundup Ready creeping bentgrass are associated with specific materials and labor that will be altered or eliminated with annual bluegrass absent from the turfgrass stand. These include applications of herbicides, growth regulators, insecticides, and fungicides; and water management.

4.2.3.1. Fumigants for establishment of new creeping bentgrass stands

A critical step in excluding annual bluegrass from creeping bentgrass is the initial establishment of a pure stand. This challenge is nearly impossible to meet without the use of fumigants because annual bluegrass has a large seed bank in soil, it germinates simultaneously with creeping bentgrass plantings, and there are no preemergence herbicides labeled for new bentgrass seedings that will control annual bluegrass. These factors mean that most new bentgrass plantings will potentially suffer some annual bluegrass encroachment from the outset.

Soil fumigants are volatile gases that kill all living organisms in biologically active soil and then dissipate into the atmosphere, leaving no residual soil activity (Harrison, 1990). They can be applied prior to new turfgrass seedings where annual bluegrass is known to be a pest. The strategy is to establish a pure stand of creeping bentgrass without initial competition and then aggressively manage the stand to prevent annual bluegrass colonization. Soil fumigants also eliminate insects, diseases, and nematodes from the soil prior to turfgrass establishment, although the natural mobility of these pests makes infestation inevitable and reduces the impetus to fumigate for these pests alone (Unruh, 2001).

The predominant fumigation product to date has been methyl bromide because of its unparalleled performance. Alternatives to methyl bromide include dazomet (Basamid®); 1,3-dichloropropene (Telone®); and various brands of chloropicrin and metam-sodium. However, none of these products alone or in combination are as an effective as methyl bromide at controlling weed seed germination (Unruh, 2001). Of these alternatives, Basamid is currently marketed to the trade for fairway and greens fumigation.

Methyl bromide is a highly toxic and technically complicated product that must be applied by a specialized contractor who injects the industrial gas into the soil under a plastic tarp. While methyl bromide is a relatively inexpensive product, the operation is quite expensive. A recent fumigation of greens in Illinois had an applied cost of more than $8,200 per acre (Mumper, 2001). More importantly, methyl bromide is scheduled for commercial phase-out in 2005 (Unruh, 2001) because it is a suspected ozone-depleting chemical (EPA, 1995).

The expense, complexity, and hazards of applying methyl bromide have historically precluded its use for fairway establishment except in the most extreme circumstances. Nearly 100% of new or renovated greens are fumigated with methyl bromide in the

southern US, but this frequency decreases to 10% or less in the far north (Zontek, 2000). Fumigation of converted agricultural soils may be less critical than renovated golf land because annual bluegrass seed is more likely to be in soils on an existing golf course.

Basamid® is a granular product that performs best when incorporated through tillage and/or thorough irrigation, as well as tarping. It does not require a specialized contractor if a tarp is not used, and costs about $1600 per acre when custom applied (Mumper, 2001). This expense is still prohibitive to many golf operations for fairway use of Basamid, but a growing number of golf course development professionals and superintendents are considering its use. If nothing else, this expensive trend is an indication of the importance the golf industry assigns to keeping annual bluegrass out of fairway turf.

A recent study of over-the-top (unincorporated) Basamid efficacy for prevention of annual bluegrass seed germination showed that control ranged between 90 and 97 percent and was improved by the use of a tarp (Park and Landschoot, 2001; Landschoot, 2001). The $1,600 per acre cost of Basamid referenced earlier did not include tarping the fairways, so fumigation performance comparable to methyl bromide may require additional investment.

The use of Roundup Ready creeping bentgrass in new fairway (and eventually greens) plantings will completely eliminate the need for fumigants when annual bluegrass is the target pest. A three-application-per-year Roundup ® program ($63 per acre applied cost), initiated four weeks after seedling emergence, will eliminate annual bluegrass before its competitive nature compromises the creeping bentgrass stand. In those cases where fumigation targets include insects or pathogens, fumigants may still be utilized. This is particularly true for nematodes that are not controlled by conventional insecticides. However, it is questionable whether superintendents will incur the expense of Basamid on fairways when conventional, non-injurious herbicides, fungicides, and insecticides can do the same job at a fraction of the cost.

4.2.3.1 Plant growth regulators (PGRs)

The uses of PGRs on golf courses are manifold and contribute to both annual bluegrass and creeping bentgrass management. They are key elements of managing mixed stands and can be employed to selectively encourage or discourage annual bluegrass vigor according to the short-term priorities of the golf course. Table 6 lists the predominant products used on golf courses and their specific management utility.

The introduction of Roundup Ready creeping bentgrass will largely eliminate at least two summer applications of paclobutrazol for bentgrass conversion on fairways. Some superintendents may choose to retain the product for its turfgrass quality enhancing traits, but Primo tends to be the product of choice for this purpose in the absence of annual bluegrass (Dernoeden, 2001; Watkins, 2001; Watschke, 2001).

Until cultivars selected for greens use are developed, Embark T&O applications should not be affected. However, Embark T&O can potentially be eliminated once appropriate

Table 6. PGRs and their applications for creeping bentgrass and annual bluegrass management

PRODUCT	UTILITY
Primo (trinexapac-ethyl), Proxy (ethephon), Cutless (flurprimidol)	Reduce mowing frequency, improve overall turfgrass condition
Embark T&O (mefluidide)	Suppress annual bluegrass seedheads and improve vigor on greens
TGR, Turf Enhancer , Trim-It (paclobutrazol); some Cutless (flurprimidol) use	Suppress annual bluegrass vigor on fairways for bentgrass species conversion

SOURCE: WATSCHKE, 2001; DERNOEDEN, 2001

cultivars become available for greens and annual bluegrass is eliminated. In addition to the reduced labor and pesticide use benefits of this change, the bentgrass phytotoxicity that is commonly associated with Embark will disappear.

4.2.3.2 Insecticides

The annual bluegrass, or Hyperodes, weevil (*Listronotus anthracinus* Dietz) is the primary insect pest that attacks annual bluegrass but not creeping bentgrass (Beard, 1982; Shetlar, 1995). Annual bluegrass weevil is primarily restricted to the northern Mid-Atlantic and New England states and is centered in the New York City metro area (Vittum, 1999), but there is a high concentration of golf courses that may be affected in this region. Annual bluegrass weevil is controlled by a wide variety of organophosphate, synthetic pyrethroid, and carbamate insecticides (CRC Press, 1998). The elimination of annual bluegrass can eliminate one spring insecticide application per season (Vittum, 2001).

4.2.3.3 Fungicides

Fungal pathogens are some of the most troublesome pests on golf course turf. Intensive irrigation, fertilization, and mowing combined with play-related traffic make the turfgrass more susceptible to diseases than in nearly any other cultural setting. The topic is far too complex to summarize here. More than twenty pathogens are known to collectively attack either creeping bentgrass or annual bluegrass (Dernoeden, 1995). Most of these pathogens will infest either turfgrass species, so elimination of one turfgrass does not necessarily reduce the need to treat many of these diseases. Others are specific to one of the two species or are a much more significant problem for one than the other.

Anthracnose (*Colletotrichum graminicola* (Ces.) Wilson), bacterial wilt (*Xanthomonas campestris* Pammel) and summer patch (*Magnaporthe poae* Landschoot and Jackson) are

three diseases that are more pervasive in (anthracnose) or specific to annual bluegrass than creeping bentgrass (Dernoeden, 2001).

Three factors affect the ability of a specific fungal pathogen to invade turfgrass – the presence of the fungus, a susceptible host, and environmental conditions (generally moisture and temperature) that promote the growth and spread of the fungus. These same factors will drive the choice of fungicide(s) and the application strategy (Dernoeden, 1995). Under severe conditions, fungicides may be applied to putting greens in tank mixes or in rotation on a 7 to 28 day schedule throughout the season to control various pathogens (Dernoeden, 1995; CRC Press, 1998). Fairways are less likely to receive the same intensity of disease pressure or management as greens.

Practically, it is difficult to predict the impact on fungicide use of eliminating annual bluegrass from creeping bentgrass fairways and greens. Neither anthracnose nor summer patch are uncommon diseases on annual bluegrass, so their elimination or reduction should eliminate some fungicide use. However, the various fungicides labeled for anthracnose and summer patch are not active solely on these two pathogens and they will be utilized to manage other diseases. Dernoeden (1995) lists more than twenty disease pathogens that attack creeping bentgrass. The use of fungicides on Roundup Ready creeping bentgrass that is free of annual bluegrass will be driven by pressure from these diseases in any given season.

A variety of fungicides control these diseases and can be used in rotation or tank mixes to minimize fungicide resistance (CRC Press, 1998). Weekly fungicide applications may be required in worst-case conditions, but such intensity is generally reserved for greens rather than fairways (Dernoeden, 2001). It is likely that at least one or two fairway treatments will be avoided for each of these diseases with the elimination of annual bluegrass and the associated incidence of these diseases.

Bacterial wilt is a recently emerging disease problem in annual bluegrass grown on putting greens, so its treatment is probably sporadic. Bacterial wilt can be suppressed (but rarely controlled) only with copper hydroxide. Copper hydroxide is potentially phytotoxic to creeping bentgrass and must be used with caution.

4.2.3.3.1 Perennial ryegrass and Gray Leaf Spot

A trend towards conversion of Kentucky bluegrass (*Poa pratensis* L.) fairways to highly improved perennial ryegrass (*Lolium perrene* L.) developed in the Mid-Atlantic States in the 1980s (Dernoeden, 1997; Vermuelen, 2000; Bonos et. al., 2000). This choice was a response to the challenges of managing the annual bluegrass and patch disease infestations that were stimulated by lowered mowing heights. After nearly a decade of impressive performance, many of these perennial ryegrass fairways were devastated between 1995 and 1998 by a fast-moving infestation of gray leaf spot (*Pyricularia grisea* Cooke) that apparently originated in the southern United States, (Schmitz, 1999; Vermeulen, 2000). Pathologists and superintendents have subsequently learned to manage gray leaf spot in fairways, but only at great expense (Dernoeden, 2001).

Golf course superintendents were largely unprepared for this development when it began and there were few effective fungicides available to mitigate the disease. Repeat applications of azoxystrobin, thiophanate-methyl, trifloxystrobin, and tank mixes of several less efficacious fungicides were used to salvage affected areas, but significant acres of perennial ryegrass fairways were lost during this period (Vermeulen, 2000). Ongoing research indicates that preventative applications are more reliable than curative due to the aggressive nature of the organism (Vincelli, 2000). The most effective products for management of gray leaf spot are also some of the most expensive – one golf course superintendent reported spending $25,000 to treat fairways with azoxystrobin followed by chlorothalonil about ten days later (Schmitz, 1999). Many golf course superintendents have to use these fungicides aggressively for extended periods to control gray leaf spot.

As a result of these costly events and the associated loss of golf revenue, golf course superintendents and club managers are rethinking their choice of turfgrass species, and many are either converting or considering the conversion to bentgrass fairways (Vermeulen, 2000; Dernoeden, 1997) or back to newer, improved Kentucky bluegrass (Bonos et al., 2000). Roundup Ready creeping bentgrass will provide an attractive environmental and economic benefit to these individuals, given that one original impetus for conversion to perennial ryegrass was an inability to satisfactorily manage annual bluegrass in Kentucky bluegrass fairways.

4.2.3.4 Syringing and hand watering

During periods of heat and/or drought stress, annual bluegrass in both greens and fairways will decline from stress before the more tolerant creeping bentgrass. Hence, nightly or early morning irrigations often are required to keep annual bluegrass from declining in the summer. Furthermore, superintendents counter midday heat stress symptoms by 'syringing', i.e., cooling the turfgrass by wetting the leaves with a light irrigation, using either manual or automated equipment or by deeper hand watering on a prescription basis.

Establishment of a pure creeping bentgrass stand will not completely eliminate the need for supplemental irrigation and/or syringing, but will reduce the frequency of the practice. While reduced water consumption is an obvious benefit, it is relatively insignificant in the context of the total seasonal irrigation demands for a typical golf course. The larger benefit will be reduced labor - one man-day per 18 holes is a common investment for this activity - during the months of July and August. While superintendents can utilize automated irrigation for syringing, prescription hand watering remains a common practice because there are significant localized turfgrass management benefits (Brame, 2001).

4.2.4 Improved performance of creeping bentgrass

An indirect but important benefit of eliminating annual bluegrass from creeping bentgrass fairways is the ability to optimize turfgrass management - including fertilization, irrigation, cultivation, pest management, and overseeding - for the sole benefit of creeping bentgrass, rather than juggling priorities for two species. These benefits were described earlier in Table 5. The result will be to improve the year round condition of the creeping bentgrass.

Perhaps the most important effect is the increased opportunity to aerate and over-seed creeping bentgrass in spring and late summer. Core aeration and verticutting are important not only as components of over-seeding operations, but also as steps to reduce thatch and soil compaction (Beard, 1982; Dernoeden, 1997). However, soil disturbance also disturbs annual bluegrass seed and, without effective annual bluegrass controls, promotes its germination coincidently with creeping bentgrass (Watschke, 1995). The herbicide, ethofumesate, provides some utility for this purpose but the program is technically challenging (Dernoeden, 2000; CRC Press, 1998; Dernoeden, 1997). Annual bluegrass control with Roundup herbicide eliminates this concern and provides opportunities to maximize creeping bentgrass density, playability, and overall quality throughout the growing season.

Experienced golfers will benefit from the elimination of annual bluegrass from mixed stands with creeping bentgrass because a lower cutting height generally can be maintained without the problems associated with annual bluegrass encroachment. Closely mowed fairway turf provides a better ball lie for the experienced golfer. The result is an overall increase in playability of the surface and competitive performance. Improved playability translates to a more rounds played and a more competitive golf business in an increasingly crowded golf course industry.

4. PUBLIC PERCEPTIONS OF THE GOLF INDUSTRY

The introduction of Roundup Ready creeping bentgrass should enhance the environmental reputation and public acceptance of new golf course construction. This author's extension pesticide education activities and professional experience in supporting and reviewing numerous golf course development projects indicate that the public is concerned about the perceived level of pesticide use on golf courses. That observation is further supported by considerable coverage of this issue in the popular press.

Extensive golf course development activity in the northeastern US during the 1990s led to frequent public debates about land use and the environmental impact of the industry (Maker, 1998; Murphy, 1997; LeDuff, 1999; Lomuscio, 1997). Pesticide usage levels and associated toxicity and environmental effects were the most common concerns of activists, particularly contamination of drinking water. There also is a perception that golf courses use larger quantities of more toxic products than other turfgrass or conventional crop management systems (Office of the New York State Attorney General,

1995; Texas Pesticide Information Network, 2001; Twyman, 2001; Cox, 1991; Putt, 1999; Mattingly, 1996).

While Roundup herbicide also is a registered pesticide, its home and garden positioning in the consumer marketplace suggests that consumer acceptance of and comfort with the product is very high. The prospect of significantly reducing uses of other, less familiar pesticides exhibiting less favorable environmental and human health profiles can contribute significantly to improving the image of the industry.

5. QUANTITATIVE ASSESSMENT OF ROUNDUP-READY CREEPING BENTGRASS BENEFITS

Quantifiable benefits, given expected cost and adoption of Roundup Ready creeping bentgrass, are presented here. We concentrate predominantly on the environmental and financial benefits of avoiding pesticide use. The following summary considers a 50% adoption rate of the technology for new seedings and renovations, and realistic estimates for frequencies of individual management practices being replaced based on site-specific needs. Previous experience with transgenic crop commercialization suggests that 50% is not an unrealistic market adoption level within a few years of introduction.

This analysis considers three benefits assessment scenarios: (1) turfgrass establishment, either for new construction or renovation of existing facilities; (2) annual maintenance operations; and (3) special cases that warrant consideration but may not constitute the norm in a typical year. Cost estimates for scenarios 1 and 3 are based on labor and material expenses as reported by three golf course superintendents (Watkins, 2001; Putnam, 2001; Mumper, 2001). For scenario 2, University scientists from Pennsylvana State University (Dr. Tom Watschke), the University of Illinois (Dr. Bruce Branham) and Purdue University (Dr. Zac Reicher) were also consulted. Application rates are based on product labels and personal communications with superintendents and university extension specialists.

Roundup Ready creeping bentgrass can theoretically be planted either as a routine over-seeding operation or for new turfgrass establishment. The logical expectation is that golf course managers will choose to employ the technology primarily for new establishments, given that only pure stands can realize the full environmental, economic, and performance benefits associated with the use of Roundup herbicide. Thus, while course-specific benefits are derived from simple acreage and management assumptions, industry-wide benefits analyses must consider levels of new construction and reconstruction activities and of the long term adoption rate of Roundup Ready creeping bentgrass.

The National Golf Foundation tracks golf course development projects throughout the US on a continual basis. There is wide recognition in the golf and real estate industries that golf course construction and renovation grew rapidly in the 1990s (Hirsch, 2001) and construction activity continues at a brisk pace today. New and reconstructed 18-hole equivalent golf course openings grew nationally from 262 to 327 between 1993 and 1998,

an increase of 25% for the period (National Golf Foundation, 1999). That figure increased another 16% to 379 in 1999 (Ratcliff, 2000) and 38% more to 524 in 2000 (National Golf Foundation, 2001). As of September 30, 2001, over 328 eighteen-hole equivalents opened, more than 560 were under construction, and 788 were in various stages of planning (National Golf Foundation, 2001). This pace suggests 438 course openings for 2001.

A review of state-by-state details in the National Golf Foundation (2001) report reveals that approximately 196 of the new openings, 314 of the ongoing construction projects, and 470 of the planned golf courses occur in those parts of the country where creeping bentgrass is routinely grown. These data suggest that with approximately 5,000 acres of fairway openings in those regions annually, the opportunity to realize the environmental and economic benefits of the technology is large relative to the size of the industry. Moreover, the National Golf Foundation acreage estimates should be considered conservative because they do not capture simple turfgrass renovations that are accomplished through reseeding without redesign or re-grading of the golf hole(s).

6.1. Environmental benefits

The primary environmental benefit of Roundup Ready creeping bentgrass will be fewer pounds of applied pesticides for weed control, annual bluegrass suppression, and disease management (see sections 4.3.2.4 and 4.3.2.4.1 and Tables 7, 8, and 9). Application rates are taken from product labels (CRC Press, 1998; Scotts, 1999; Great Lakes Chemical Corporation, 1995).

The major potential input reduction during establishment is the elimination of fumigants, specifically methyl bromide for greens and Basamid for fairways. For routine annual maintenance after grow-in, further benefit can be realized from the replacement of multiple herbicide and PGR applications with Roundup herbicide, and the elimination of certain fungicide and insecticide applications. Special considerations include Basagran treatments for yellow nutsedge and fungicide use impacts from converting from perennial ryegrass to Roundup Ready creeping bentgrass.

6.1.1 Turfgrass establishment

Potential fumigant reductions achievable with the adoption of Roundup Ready creeping bentgrass are presented in Table 7. In the absence of actual use data, the following assumptions were applied to use estimates in this analysis:

- Each treatment covers all eighteen holes, or 23 acres for fairways and 2.1 acres for putting greens (Doane Marketing Research, Inc., 1999).
- Tees are ignored here because acreage data were not available for analysis, but creeping bentgrass tees are not uncommon and will add to the potential benefits. However, tee acreage on golf courses is similar to that of greens, so benefits may be similar.

- More than 280 golf courses annually in the US plant putting greens to creeping bentgrass. This represents 70% of the more than 400 golf courses opening annually in the US, based on the combined Doane's (1999) and National Golf Foundation (2001) data.
- More than 140 golf courses (50% of 280 from the previous bullet) annually in the US fumigate new creeping bentgrass greens, historically with methyl bromide but recently with Basamid as an alternative. This 50% fumigation frequency is based on a gradient of nearly 100% fumigated greens in southern tier states to <10% fumigated greens in northern tier states (Zontek, 2001).
- Approximately 100 golf courses annually in the US plant fairways to creeping bentgrass. This represents 50% of 200 golf courses that open annually in creeping bentgrass growing areas of the northern US (National Golf Foundation, 2001).
- Approximately 15 golf courses (15% of 100 from the previous bullet) annually in the US will fumigate new creeping bentgrass fairways with Basamid. This 15% fairway fumigation frequency is half of the estimate for greens established between the warm-season/cool-season transition zone (Maryland-to-Kansas transect) and the Canadian border, based on the gradient described in the previous bullet point. Basamid is a cheaper alternative to methyl bromide that will encourage more frequent fairway fumigation, but the frequency is unlikely to ever approach that of greens. This trend will take a number of years to develop while superintendents gain experience with Basamid.
- Approximately 70 new golf courses will replace greens fumigation with a Roundup herbicide program, and eight will do so for fairways. These estimates are based on a 50% market penetration of Roundup Ready creeping bentgrass for new plantings.

Assuming 50% market penetration of newly constructed fairways and putting greens, adoption of Roundup Ready creeping bentgrass can replace approximately 127,000 pounds of annual fumigant use. The fumigant reduction is almost evenly divided between methyl bromide and dazomet. Overall, this is a conservative estimate of fumigant reduction because additional conversions occur that are not tracked by the National Golf Foundation.

Table 7. Turfgrass establishment fumigant reductions resulting from adoption of Roundup Ready creeping bentgrass

TURFGRASS ESTABLISHMENT ACTION	ACTIVE INGREDIENT PER ACRE	INDIVIDUAL COURSE IMPACT	ANNUAL MARKET PENETRATION	ANNUAL INDUSTRY IMPACT
Potential Basamid use on all US fairways	350 lb	8,050 lb	100 golf courses	805,000 lb
Estimated Basamid use on bentgrass fairways	350 lb	8,050 lb	15 golf courses[1]	120,750 lb
Potential Basamid use reduction			*8 golf courses[2] (53% reduction from fumigated bentgrass fairways)*	*64,400 lb (8% reduction from all fairways fumigated in US)*
Roundup applications	3 x 1.5 lb	104 lb	8 golf courses[2]	828 lb
Potential pesticide use reduction on fairways		*7,946 lb*		*63,572 lb[3]*
Estimated methyl bromide use on all US putting greens	300 lb	630 lb	200 golf courses	126,000 lb
Estimated methyl bromide use on creeping bentgrass greens	300 lb	630 lb	140 golf courses[4]	88,200 lb
Potential MeBr use reduction on greens			*70 golf courses[5] (50% reduction from fumigated bentgrass greens)*	*63,000 lb (35% reduction from all greens fumigated in US)*
Roundup applications	3 x 1.5 lb	9.5 lb	70 golf courses[5]	662 lb
Potential pesticide use reduction on greens		*886 lb*		*62,338 lb[6]*

[1] 15% Basamid® use x 200 courses built in growing area x 50% planted to bentgrass fairways
[2] 50% replacement of estimated Basamid fumigations with Roundup program
[3] 50% of estimated Basamid use minus anticipated Roundup replacement
[4] 50% MeBr fumigation x 400 courses built in US x 70% bentgrass greens
[5] 50% replacement of estimated MeBr fumigations with Roundup program
[6] 50% of estimated MeBr use minus anticipated Roundup use

6.1.2. Annual (post grow-in) maintenance

Potential pesticide use reductions achievable with the adoption of Roundup Ready creeping bentgrass are presented in Table 8. In the absence of actual use data, Monsanto and The Scotts Company collaborated with University turf scientists from Pennsylvana State University (Dr. Thomas Watschke), Purdue University (Dr. Zachary Reicher) and the University of Illinois (Dr. Bruce Branham) to develop hypothetical estimates of fungicide, herbicide and plant growth regulator reduction under an established Roundup Ready creeping bentgrass stand. The estimates of pesticide reduction were obtained via an interview approach with each collaborator.

Each scientist was asked to categorize the typical management approaches utilized on existing creeping bentgrass golf courses. Each scientist quantified the fungicides, plant growth regulators (PGR) and herbicides in the above management categories and estimated reductions of those pesticides for an established sward of Roundup Ready creeping bentgrass.

Two main management categories surfaced with the all three scientists:

- Courses with the objective of maintaining annual bluegrass, remain status quo, or maintain an ecologically stable bent/Poa mix.

- Courses with the objective of reducing stands of annual bluegrass or keep *P. annua* in check, or maintain as pure a stand of creeping bentgrass possible.

The following results reflect a comparison between these two management categories and the current traditional creeping bentgrass applications required were a pure stand of Roundup Ready creeping bentgrass established.

Other assumptions or data utilized in the analysis include:

- Fairways were approached independently from greens and/or tees
- Greens and tees were combined as a single and independent approach from fairways
- Doane (1999) pesticide usage data for creeping bentgrass greens, tees, and fairways was employed.
- Market penetration was expressed as a percent of the 1999 existing creeping bentgrass acreage cited by Doane (1999).
- In addition, as described in Section 4.3.2.3, Vittum (2001) estimates that at least one insecticide application is made to control the annual bluegrass weevil because of annual bluegrass encroachment onto creeping bentgrass fairways in a highly localized area of ca. a 100 mile radius around New York City, NY. This represents approximately 2,600 acres of putting greens and 5,600 acres of fairways. The pesticide reduction estimates provided by Drs. Branham, Reicher and Watschke did not encompass a reduction in insecticide use for annual bluegrass weevil control.

The reduction in the use of fungicides, herbicides, insecticides and PGRs is presented in Table 8. These estimates represent the reduction in these four pesticides for one18 hole golf course. If these averages were then applied to the total number of AI pounds of fungicides, herbicides, insecticides and PGRs utilized on the 11,809 golf courses with creeping bentgrass greens/tees and/or fairways, an estimated 415,083 pounds would be eliminated. Excluding the niche use of insecticides, the overall average reduction in combined AI of herbicides, fungicides and PGRs is estimated to be 20%.

Table 8. Routine pesticide use reductions resulting from adoption of Roundup Ready creeping bentgrass on golf course fairways, putting greens and tees[1].

ANNUAL PESTICIDE TREATMENT	CURRENT ACTIVE INGREDIENT APPLIED (lb)[2]	POTENTIAL PERCENT REDUCTION W/RRCB[3]	ACTIVE INGREDIENT REDUCED (lb)	ACTIVE INGREDIENT REDUCED PER COURSE[4] (lb)
Herbicide	G&T 79,486 Fairways 48,296 Total 127,782	G&T 0.74 Fairways 0.35	G&T 58,820 Fairways 16,904 Total 75,723	G&T 3.93 Fairways 7.35 Total 11.28
Fungicide	G&T 1,145,823 Fairways 929,971 Total 2,075,794	G&T 0.14 Fairways 0.16	G&T 160,415 Fairways 148,795 Total 309,211	G&T 10.73 Fairways 64.77 Total 75.5
Plant growth regulator	G&T 6,857 Fairways 14,091 Total 20,948	G&T 0.69 Fairways 0.64	G&T 4,731 Fairways 9,018 Total 13,750	G&T 0.31 Fairways 3.92 Total 4.23
Insecticide[5]	G&T 95,911 Fairways 61,469 Total 157,380	G&T 0.05 Fairways 0.18	G&T 5,200 Fairways 11,200 Total 16,400	G&T 0.30 Fairways 4.87 Total 5.17
TOTAL	G&T 1,328,077 Fairways 1,053827 Total 2,381,904		G&T 229,166 Fairways 185,917 Total 415,083	G&T 15.27 Fairways 80.91 Total 96.18

1. Courses that may decide to renovate their existing turfgrass in favor of Roundup Ready creeping bentgrass are not accounted for in Table 8.
2. Estimated pesticide usage (Doane, 1999)
3. Based on estimates provided by University turf scientists
4. Based on 14,940 estimated courses with creeping bentgrass greens and/or tees and 2,297 courses with creeping bentgrass fairways (Doane, 1999)
5. Represents the potential reduction of insecticide on 2,600 acres of putting greens and 5,600 acres of fairways for control of annual bluegrass weevil, which is estimated to be a pest in a highly localized area representing approximately a 100-mile radius around New York City, New York (Vittum, 2001).

Although Monsanto and The Scotts Company do not expect 100% of the existing creeping bentgrass golf courses to switch to Roundup ready creeping bentgrass, the adoption of other Roundup Ready crops would justify a 50% adoption level. Table 9 reflects the pesticide AI reduction possible at 25%, 50%, 75%, and 100% Roundup Ready creeping bentgrass penetration levels.

Table 9. Pesticide active ingredient (AI) reduction under a Roundup Ready creeping bentgrass system at various levels of market penetration.

Market Penetration	**AI Reduced (lbs)**
100%	415,083
75%	311,312
50%	207,542
25%	103,771

6.1.3. Specialty applications

Potential pesticide use reductions from two specialized considerations are shown in Table 10. The specialty nature of these applications makes estimating industry-wide impacts inappropriate. However, the current industry troubles with gray leaf spot on perennial ryegrass and historical problems with Kentucky bluegrass suggest a high probability that golf courses will consider adopting (Roundup Ready) creeping bentgrass as an alternative species.

Table 10. Specialty pesticide use reductions on fairways from adoption of Roundup Ready creeping bentgrass

SPECIALTY PESTICIDE FAIRWAY APPLICATION	RATE/A	INDIVIDUAL COURSE REDUCTION PER APPLICATION
Basagran application for yellow nutsedge control	1 lb	23 lb
Azoxystrobin fungicide application for gray leaf spot control on perennial ryegrass	0.55 lb	12.7 lb

With hundreds of golf courses in the Mid-Atlantic and Midwest states with perennial ryegrass fairways affected by gray leaf spot, the potential for bentgrass conversion and hence fungicide reduction is high. It is important to note that fungicide applications for gray leaf spot are generally made preventatively and routinely, so a single application

will probably be less than the normal reduction realized by a conversion from perennial ryegrass to creeping bentgrass.

6 CONCLUSION

Golf course managers face a myriad of agronomic challenges that stem from the exacting quality and performance demands of the game combined with the added stresses of traffic and play. The adoption of Roundup Ready creeping bentgrass has the potential to become the heart of an improved agronomic management system. As identified in this analysis, this system could eliminate the use of nearly one million pounds of pesticides, which includes other herbicides, fumigants, fungicides, insecticides and plant growth regulators. Although, there is no evidence to suggest that pesticides cannot be used safely on golf courses, the use of Roundup herbicide to control weeds on Roundup Ready creeping bentgrass fairways and greens offers an opportunity to significantly decrease overall pesticide use and thus reduce the risks associated with comparatively more hazardous products.

Finally, the aggressive development of golf courses throughout the United States during the 1990s has increased competition in most regional golf markets. The use of Roundup herbicide on Ready creeping bentgrass has the potential to save the golf course industry several million dollars through reduced pesticide use. In addition to lower costs, the quality of the turf will also improve, which may increase the number of rounds played by golfers. All of these benefits will contribute to a better financial performance for the individual golf course and the golf industry as a whole.

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Appendix A. Table 1. Key Weed Control Products Used on Golf Courses

Product brand	Active	Label signal word	Restrictions	Max lbs/acre (single treatment) lb. a.i./a	Max lbs/acre (yearly) lb. a.i./a	Label warnings and special directions
Roundup Pro	glyphosate	Caution	NS	0.75	8.0	Causes eye irritation; do not store spray solution in steel
Proturf Goosegrass / Crabgrass Control	bensulide / oxadiazon	Warning	Do not use on parks, recreation areas or other public areas.	6.0 / 1.5	12.0 / 3.0	Harmful if swallowed; substantial but temporary eye injury; toxic to fish and aquatic invertebrates; highly toxic to bees; may impare reproduction in birds if used during breeding season; immediate watering-in required
Ronstar G	oxadiazon	Warning	Do not allow domestic animals to graze treated areas	4.0	NS	Harmful if inhaled; toxic to fish; product should not be used…where irrigation or rainfall results in…contamination of surface waters through…runoff; product should not be used on putting greens or tees
Dimension Turf and Ornamental	dithiopyr	Warning	Do not graze livestock or feed forage cut from treated areas	0.5	1.5	Harmful is swallowed; substantial but temporary eye injury; may cause allergic skin reaction; toxic to fish and highly toxic to other aquatic organisms; dirft and runoff may adversely affect aquatic organisms; numerous warning for turf injury
Pendulum 3.3EC	pendimethalin	Caution	NS	3.0	NS	Harmful if absorbed through skin; toxic to fish; runoff hazardous to aquatic organisms; activation by rainfall necessary for optimum weed control; delay reseeding for 3 months and sprigging for 5 months after appl.
Millenium Ultra	2,4-D / clopyralid / dicamba	Danger	NS	0.56 / 0.07 / 0.07	1.12 / 0.14 / 0.14	Corrosive; irreversible eye damage; Harmful if swallowed or absorbed through skin; toxic to aquatic invertebrates; runoff may be adversely affect aquatic invertebrates and nontarget plants; clopyralid may leach to groundwater
Barricade 65WG	prodiamine	Caution	Do not graze or feed forage from treated areas; do not use on putting greens	1.5	1.5	Harmful if inhaled or absorbed through skin; may cause allergic reactions; may be toxic to fish; drift and runoff from treated areas may be hazardouse to aquatic org.; rotation restrict. of 1 yr for ornamentals not labeled unless strip test shows species safety; rainfall needed within 14 days; numerous turf injury warnings
Acclaim Extra	fenoxyprop	Caution	NS	0.16	0.48	Toxic to fish and aquatic invertebrates; numerous warning statements for turf injury under various conditions; not for use on putting greens
Turf Enhancer	paclobutrazole	Caution	NS	0.75	2.25	Harmful if swallowed or absorbed through skin; delay sprigging for 4 weeks and sodding for 2 weeks after appl.

NS = Not Specified

Appendix A. Table 2. General Hazard Profile for Key Weed Control Products Used on Golf Courses

Active	Cancer class	Applicator / Handler PPE	Label Signal Word	Other Comments
glyphosate	**E**	Long-slv shrt, long pants, shoes, socks	Caution	
bensulide	na	Coveralls over long-slv shrt, long pants, chem resist gloves & footwear, dust respirator	Warning	Organophosphate; concerns for exposure to birds and bees; concern for occupational exposure to handlers for uses on golf courses; use restricted to golf courses and residential lawns (professional applicators)
oxadiazon	C, Possible Human	Coveralls over shrt-slv shirt, shrt pants, waterproof gloves, protect. eyewear, chem. resist. headgear & footwear	Warning	No food use tolerances
dithiopyr	na	Coveralls over shrt-slv shirt, shrt pants, socks, chem. resist. gloves, protect. eyewear, chem. resist. footwear; mixers & loaders add chem. resist. apron	Warning	
pendimethalin	C, Possible Human	Coveralls over long-slv shirt, long pants, chem. resist. gloves, shoes, socks	Caution	Concern for occupational exposure to handlers from uses in turf; chemical resistant gloves added to baseline PPE; risk mitigation to post-appl. expousure: max. appl. rate will be reduced to 2 lb/A for residential uses.
dicamba	D, not classifiable, inadequate dosing	Long-slv shirt, long pants, protective eyewear, shoes, socks, rubber gloves	Danger	EPA aggregate exposure assessment: Additional 10X uncertainty factor for FQPA applied due to increased susceptibility to offsrping in 2-gen repro study; evidence of neurotoxcity in multiple studies; developmental neurotox study needed
2,4-D	D, not classifiable, inadequate evidence	Long-slv shirt, long pants, protective eyewear, shoes, socks, rubber gloves	Danger	EPA aggregate exposure assessment: Prenatal toxicity concerns for infants and children; additional 3x uncertainty factor for FQPA applied due to developmental effects in rats with the absence of maternal toxicity
clopyralid	E, Not Likely	Long-slv shirt, long pants, protective eyewear, shoes, socks, rubber gloves	Danger	EPA aggregate exposure assessment: 3x uncertainty factor for FQPA retained due to fetal neuropathology in rabbit develop. tox study; develop. neuro. study required.
prodiamine	C, Possible Human	Long-slv shirt, long pants, waterproof gloves, shoes, socks	Caution	
fenoxaprop	Q*	Long-slv shrt, long pants, shoes, socks	Caution	Q1* quanitative risk assessment based in increase adrenal tumors in mice; referred for CARC review
paclobutrazole	na	Long-slv shirt, long pants, chem. resist. gloves, shoes, socks	Caution	

na=not available; PPE = Personal Protective Equipment

Source: Information summarized in this table was obtained from EPA Registration Standard documents, Re-registration Eligibility Decision documents, Pesticide Fact Sheets and/or Federal Register Tolerance Notices or Final Rule publications

Appendix A, Table 3. Comparison of Effects on Fish Species for Key Weed Control Products

Active	max lb ai /a (1 trtmt)	EEC* ppm	Fish LC_{50} (a.i.) range (ppm)		Fish Risk Quotient** range		Label Warnings
			low	high	worst	best	
glyphosate	0.75	0.03	85	130	0.0003	0.0002	
bensulide	6.0	0.20	0.32	1.78	0.63	0.11	Toxic to fish
oxadiazon	4	0.134	1.1	8.2	0.12	0.016	Toxic to fish
dithiopyr	0.5	0.017	0.46	2.16	0.04	0.008	Toxic to fish
pendimethalin	3.0	0.10	0.138	0.71	0.73	0.14	Toxic to fish
dicamba	0.07	0.002	28	400	0.0001	0.00001	
2,4-D	0.56	0.02	28.4	168	0.001	0.0001	
clopyralid	0.07	0.002	na	na			
prodiamine	1.5	0.05	0.45	100	0.112	0.0005	May be toxic to fish at concentrations substantially above level of water solubility
fenoxaprop	0.16	0.005	0.31	7.12	0.02	0.0008	Toxic to fish
paclobutrazole	0.75	0.03	23.6	27.8	0.001	0.001	

Aquatic LC_{50}s from EPA Ecotoxicology one-liner database

na = information not available

* EEC assumes 5% drift and 5% runoff from 10 acre field to 1 acre pond 6 feet deep

** R.Q. is EEC/LC_{50}. Bold if > 0.5 = Level of Concern [Ecological Effects, Rejection Analysis] Trigger Value of 0.5 for acute aquatic risk quotients was established in EPA's Ecological Effects Pesticide Rejection Rate Analysis document, date December, 1994, page 4.

Appendix A, Table 4. Comparison of Effects on Aquatic Invertebrates for Key Weed Controls Products

Active	max lb/a (1 trtmt)	EEC* ppm	Invertebrate EC_{50} (a.i.) range (ppm) low	high	Invertebrate Risk Quotient** range worst	best	
glyphosate	0.75	0.03	780		0.00003		
bensulide	6.0	0.20	0.062	1.4	3.25	0.14	Toxic to aquatic invertebrates
oxadiazon	4.0	0.134	0.27	15.4	0.50	0.009	
dithiopyr	0.5	0.017	0.58	17	0.03	0.001	Highly toxic to aquatic invertebrates
pendimethalin	3.0	0.10	0.28	1.6	0.36	0.06	
dicamba	0.07	0.002	56	750	0.00004	0.000003	
2,4-D	0.56	0.02	0.14	100	0.13	0.0002	Toxic to aquatic invertebrates
clopyralid	0.07	0.002	na	na			
prodiamine	1.5	0.05	0.083	2.1	0.61	0.024	
fenoxaprop	0.16	0.005	0.098	26.84	0.055	0.0002	Toxic to aquatic invertebrates
paclobutrazol	0.75	0.03	na	33.2	na	0.001	

Aquatic LC_{50}s from EPA Ecotoxicology one-liner database

na = information not available

* EEC assumes 5% drift and 5% runoff from 10 acre field to 1 acre pond 6 feet deep

** R.Q. is EEC/LC_{50}. Bold if > 0.5 = Level of Concern [Ecological Effects, Rejection Analysis]
Trigger Value of 0.5 for acute aquatic risk quotients was established in EPA's Ecological Effects Pesticide Rejection Rate Analysis document, dated December, 1994, page 4.

Appendix A, Table 5. Comparison of Effects on Aquatic Plants for Key Weed Control Products

Active	max lb/a (1 trtmt)	EEC* ppm	Aquatic Plant EC_{50} (a.i.) range (ppm) low	high	Aquatic Plant Risk Quotient** range worst	best	
glyphosate	0.75	0.03	0.9	39.9	0.028	0.001	
bensulide	6.0	0.20	na	na	na	na	
oxadiazon	4.0	0.134	0.004	0.126	33.6	1.1	
dithiopyr	0.5	0.017	0.02	na	0.84	na	
pendimethalin	3.0	0.10	0.005	0.174	20.2	0.58	May adversely effect endangered species of non-target plants
dicamba	0.07	0.002	0.05	3.7	0.05	0.001	
2,4-D	0.56	0.02	0.58	188.5	0.032	0.0001	
clopyralid	0.07	0.002	na	na			Run-off may adversely affect non-target plants
prodiamine	1.5	0.05	na	na			
fenoxaprop	0.16	0.005	0.43	431.9	0.013	0.00001	
paclobutrazol	0.75	0.03	na	na			

Aquatic LC_{50}s from EPA Ecotoxicology one-liner database

na = information not available

* EEC assumes 5% drift and 5% runoff from 10 acre field to 1 acre pond 6 feet deep

** R.Q. is EEC/LC_{50}. Bold if > 0.5 = Level of Concern [Ecological Effects, Rejection Analysis]
Trigger Value of 0.5 for acute aquatic risk quotients was established in EPA's Ecological Effects Pesticide Rejection Rate Analysis document, dated December, 1994, on page 4.

Appendix A, Table 6. Comparison of Environmental Parameters for Weed Control Products

Actie	Field Soil Dissipation DT_{50} (days)		Soil Binding Koc		Ground Water Ubiquity Score [1]		Comments
	range	nominal value [2]	range	nominal value [2]	(GUS)	Class	
glyphosate	2 - 142	15	1823-11,667	5500	0.3	L	Glyphosate parameters from MRIDs 108192, 42607501 as cited in the RED
bensulide	3-180	120	740-7000	3900	0.85	L	
oxadiazon	30-180	75	1600-5300	3445	0.87	L	
dithiopyr	3-14	17	na	1638	0.97	L	
pendimethalin	8 - 480	174	5000-29000	13400	-0.28	L	
dicamba	8 - 25	16	7 - 21	13	3.5	H	Label warnings for leaching to ground water
2,4-D	4-15	14	20-530	48	2.7	M	Label warnings for leaching to ground water
clopyralid	10-30	13	na	36	2.7	M	Label warnings for leaching to ground water
fenoxaprop	9.4-14	12	9490-53700	9490	0.02	L	Label warning to avoid conditions which favor runoff
paclobutrazol	7-973	200	144-945	500	2.99	H	

[1] GUS: Low (L) leaching Potential GUS < 1.8
Moderate (M) Leaching Potential 1.8 < GUS < 2.8
High (H) Leaching Potential GUS > 2.8

Data taken from USDA ARS Pesticide Properties Database at http://www.arsusda.gov/ppdb.html

[1] Gustafson, D.I. (1989) Ground Water Ubiquity Score: A Simple Method for Assessing Pesticide Leachability. *Journal of Experimental Toxicology and Chemistry,* **8**, pp. 339-357.

[2] Nominal value refers in most cases to the "suggested value" in the USDA Pesticide Properties Database.
For glyphosate, "nominal value" refers to the mean value taken from MRIDs108192 or 42607501 cited in the RED.
na = information not available in the USDA Pesticide Properties database

Appendix II

Predicted Impact of Transgenic, Herbicide-Tolerant Creeping Bentgrass Turf on Water Quality in Water Bodies Adjacent to Golf Courses

Bruce E. Branham, Katherine H. Carr, Tamara L. Estes, David I. Gustafson, Christophe Gustin, Scott Harrison, Richard L. Morris, Eric Nelson and Terry B. Stone

Predicted Impact of Transgenic, Herbicide-Tolerant Creeping Bentgrass Turf on Water Quality in Water Bodies Adjacent to Golf Courses

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ABSTRACT

Golf courses in the United States are treated by a large number of weed, insect, and disease control chemicals. In a recent year (1999), the 35,000 ha of creeping bentgrass (*Agrostis stolonifera*) on these courses were treated with an average of approximately 30 kg/ha of such chemicals. Although runoff from turf is much less than in most conventional cropping situations, losses are possible under heavy rainfall conditions. Small surface water bodies are often adjacent to treated portions of golf courses and may be directly contaminated by such runoff. We estimated the potential water quality impacts of such chemicals using a standard computer modeling scenario established by the US EPA. This modeling system predicts pesticide concentrations in a representative water body as a function of chemical properties, use pattern, weather, and site. We compared several pesticide application scenarios required by conventional creeping bentgrass with the reduced pesticide program required to cultivate a new variety of creeping bentgrass that has been transgenically modified to tolerate applications of glyphosate, a nonselective herbicide with favorable environmental properties. The predicted water body concentrations for all pesticides were compared with three aquatic ecological end-points: fish, aquatic plants, and invertebrates. The model results indicate that the displacement of several pesticides made possible by genetic modification of creeping bentgrass would reduce the risk of approaching ecologically toxic pesticide concentrations in water bodies adjacent to golf courses, though no currently-registered uses were predicted to exceed ecologically toxic concentrations at the 90th percentile level of assessment.

Keywords: creeping bentgrass (*Agrostis stolonifera*), glyphosate, Roundup Ready, drinking water, water quality, computer modeling, transgenic crops, surface water, PRZM-EXAMS, exposure assessment

1. INTRODUCTION

Golf courses in the United States are treated by a large number of weed, insect, and disease control chemicals. In a recent year (1999), the 35,000 ha of creeping bentgrass (*Agrostis stolonifera*) on these courses were treated with an average of approximately 30 kg/ha of such chemicals. Although runoff from turf is much less than in most conventional cropping situations,[1-5] losses are possible under heavy rainfall conditions. Small surface water bodies are often adjacent to treated portions of golf courses and may be directly contaminated by such runoff. For these reasons there is an impetus to adopt practices and technologies for golf courses that offer the promise of decreased water resource impacts. One such option now under development is Roundup Ready® creeping bentgrass (denoted hereafter as RRCB) that has been genetically engineered to withstand applications of a non-selective herbicide, glyphosate.

In this paper, computer modeling is used to assess the expectation of reduced water quality impacts, and to quantitatively compare the net impact on water quality in a representative turf scenario, if conventional creeping bentgrass were switched to the transgenic variety. We begin with a description of current weed and pest control programs in the management of creeping bentgrass on golf courses. We then discuss the herbicide glyphosate and provide a list of the pesticides whose use would be reduced when switching to the transgenic, glyphosate-tolerant creeping bentgrass (RRCB). The computer models and modeling scenario (weather, soils, production alternatives, herbicide applications) are then described. We present the predicted concentrations for glyphosate and the other pesticides and compare them with three ecotoxicological endpoints: fish, aquatic invertebrates, and aquatic plants.

2. MATERIALS AND METHODS

2.1 Conventional Pest Control Strategies in Golf Course Turf

All bentgrass turfs need a general weed control program that will vary with the climate and the severity of weed infestation. The warmer the climate in which the turf is grown, the more frequent and aggressive herbicide applications will be required. Broadleaf weeds in bentgrass are readily controlled with the use of postemergence herbicides typically from the phenoxy, benzoic, and pyridinyloxy acid families. Applications are routinely made in the spring and/or early fall. Applications during the summer are normally avoided due to the potential for bentgrass injury.
If annual grasses are a concern, a preemergence herbicide can be applied in the early spring to provide season-long control (Table 1). However, due to concerns regarding root injury by this category of herbicides, some turf managers prefer to use a postemergence grass control strategy. Postemergence applications are usually initiated in late June/ early July when grass weeds first become evident in turf. Due to concerns of bentgrass phytotoxicity with these herbicides, multiple applications of low rates of

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postemergence herbicides are often employed. Where annual grassy weed pressure is very high, both pre and postemergence herbicides may be used.

Turf managers currently must make a choice to either manage annual bluegrass as a turf or to try to eliminate it from the stand. Those turf managers who chose to manage annual bluegrass will need to apply fungicides and insecticides necessary to prevent pest damage to annual bluegrass. Those turf managers who are aggressively trying to control annual bluegrass tend to make frequent use of herbicides and plant growth regulators. Despite the name, the majority of annual bluegrass found in golf course turf has a perennial life cycle.[6] Control programs must focus on prevention of additional annual bluegrass encroachment from soil seed reservoirs, as well as the reduction in existing populations. A two-pronged approach is often used with preemergence herbicides applied to prevent establishment from seed and postemergence herbicides or plant growth regulators used to reduce existing populations. Most of the preemergence herbicides (Table 1) can be used to prevent annual bluegrass establishment from seed, although most are not registered for use on putting green height creeping bentgrass. Preemergence herbicide applications are typically made in the late summer to coincide with the primary germination window for annual bluegrass. An additional, booster application may be made in the spring to prevent spring annual bluegrass establishment.

For postemergence control, ethofumesate is the only effective, labeled product for postemergence annual bluegrass control. Because the tolerance of creeping bentgrass to ethofumesate is low, rates of 0.84 kg ai ha^{-1} or less are used. Typically, 2-3 applications are made in the fall as the turf is approaching winter dormancy. Applications made at this time are more efficacious than applications at other times of the year.
A second, more popular approach to annual bluegrass reduction is the use of the plant growth regulator, paclobutrazol. This PGR selectively inhibits annual bluegrass more than creeping bentgrass; allowing a more gradual reduction in annual bluegrass as the creeping bentgrass literally grows over the highly suppressed annual bluegrass plants.[7] Using this approach, paclobutrazol is applied at rates of 0.1 kg ai ha^{-1} and higher with applications made every two to three weeks throughout the growing season. Because turf managers value the reduction in total clippings achieved by using this approach, elimination of annual bluegrass by using RRCB will not result in the elimination of the use of PGRs, rather different PGR products will most likely be substituted for paclobutrazol.

Annual bluegrass is attacked by two pests that don't attack creeping bentgrass. The disease *Magnaporthe poae* (summer patch) will attack annual bluegrass while leaving bentgrass unscathed. Some turf managers choose not to apply fungicides for this disease as part of a strategy to reduce annual bluegrass. However, the population of annual bluegrass is often so high that treatment is necessary to avoid serious reductions in turf quality. *Magnaporthe poae* attacks the roots of annual bluegrass and because of this, curative pesticide applications are problematic since by the time evidence of the disease is noticeable on the foliage, the root systems are no longer functioning. Preventative applications are made twice per year in mid and late spring.

Finally, the annual bluegrass weevil is insect that only attacks annual bluegrass. An insecticide application would be necessary to prevent damage from this pest.

2.2 Pesticides Included in Modeling

2.2.1 Glyphosate

Glyphosate is a widely used broad-spectrum herbicide that serves as the active ingredient in many products used worldwide, including Roundup® herbicide. This general discussion of its environmental characteristics and agronomic use comes from a recently published ecological risk assessment for the Roundup formulation.[8] The key physical and chemical properties of glyphosate are given in Table 1.[8-9] Glyphosate is an amphoteric compound with both pK_a and pK_b values; the latter accounts for its very high affinity for soil binding. Glyphosate disspates readily from soil predominantly due to biodegradation, which is mediated primarily by bacteria and fungi. The geometric mean field dissipation half-life is 17 d. A conservative range of aquatic half-life values has been estimated to be from 7 to 14 d.[26]

Glyphosate-based herbicides have a broad spectrum of activity towards plants. Glyphosate stops plant growth by inhibiting the production of essential aromatic amino acids through competitive inhibition of the enzyme enolpyruvylshikimate phosphate (EPSP) synthase. This enzyme is found in all plants but not in animals, giving glyphosate a low level of toxicity to animals. Glyphosate herbicides are effective only when applied directly to the plant surface. Once applied to a plant, glyphosate is assimilated by leaves and rapidly translocated within the phloem. In agricultural uses, glyphosate is generally applied as an aqueous solution by ground sprayers. The timing and frequency of glyphosate application is dependent on the target weed species and must be within the maximum-allowed levels specified on the label. In the US, annual maximum use rates, defined as the sum of all glyphosate applications made to a given site during one year, are limited to no more than 6.73 kg a.e..ha^{-1} (acid equivalents per ha) for crops. A single application is designed to effectively kill weeds present at the time of treatment.

2.2.2 *Other Pesticides*

The other fourteen pesticides included in the modeling are listed in Table 1. The physical properties listed in this table come from a variety of sources.[8-12] Certain uses of all fifteen pesticides will be impacted to varying degrees when conventional creeping bentgrass is replaced by RRCB. In some cases, the use of the product is expected to be completely displaced by the exclusive use of glyphosate for weed control. In other cases, only a partial reduction in use is anticipated either due to limited current use of the product on bentgrass or due to the reduction arising from limited, secondary effects of the new, transgenic variety. The extent to which the use of each of the pesticides would be impacted is indicated in Table 1.

2.3 Computer modeling

2.3.1 Description of computer programs

The U.S. EPA computer models PRZM v3.12[13] and EXAMS v2.98.01[14] were combined to generate distributions of estimated reservoir exposure concentrations for each of the pesticides. PRZM is a model that simulates the movement of pesticide and water in unsaturated soil, within and below the plant root zone, and in runoff water and sediment. The EXAMS model is designed to simulate the behavior of organic chemicals in aquatic ecosystems. Meteorological (daily), hydrological, cropping, pesticide physico-chemical properties, and soils information were input into the PRZM model to generate pesticide loadings in runoff water and soil. These pesticide loadings were then used as input for the EXAMS model in conjunction with pond characteristics, meteorology, and chemical information to estimate pesticide concentrations in a so-called "standard" pond scenario developed by US EPA.[14] All modeling was performed under the principles of Good Modeling Practices described by Estes and Coody.[15]

2.3.2 Description of the geographic scenario

The US EPA has established two standard geographic scenarios (Florida and Pennsylvania) to represent the use of pesticides on turf. Due to the proposed use pattern for RRCB (primarily northeastern US) only the Pennsylvania scenario was judged to be relevant for this study. This site is located in York County in south-central Pennsylvania. The soil selected to simulate typical field conditions there is a Glenville silt loam, which is a fine-loamy, mixed, active, mesic, Aquic Fragiudults and has a drainage pattern categorized as USDA Hydrologic Group C. Weather data for this scenario are from Allentown, Pennsylvania (W14737) and are taken from the 36-year time period 1948-1983. Because golf courses in this region are typically well irrigated during the warmer seasons, irrigation was added during the months of May through September according to the following rule: for any day where simulated water loss due to evapotranspiration (ET) was greater than the natural rainfall for that day, irrigation was set equal to 80% of the net daily ET loss. This rule resulted in the addition of an average of nearly 4 cm of irrigation water during each of the two warmest months (June and July) and lesser amounts during May and in the August-September period (see Table 2). This is entirely consistent with current golf course water management practices in the northeastern US.[16]

2.3.3 Treatment scenarios

The application rates for each of the fifteen modeled pesticides were chosen equal to maximum label rate for the use pattern most likely to be affected by the planting of RRCB (see Table 3). The dates of application were chosen to be typical for use of these compounds in southern Pennsylvania. Following standard regulatory practice for the type of ecological assessment presented here, a 10 hectare area fully-treated by each pesticide was assumed to deliver its runoff to a standard receiving water body with a surface area of 1 ha and a depth of 2 m.

3. MODELING RESULTS AND DISCUSSION

The simulations generate concentrations for each day in the 36-year simulation period. In order to easily summarize an interpret this vast amount of information, attention is focused on the 90th percentile annual maximum moving average concentrations predicted in the receiving water body, for various time periods ranging from 24 hours up to 90 days (see Table 4). The time periods shown in Table 4 (24 hour, 4-, 21-, 60-, and 90-days) are the standard durations reported by the US EPA model, EXAMS, and are each chosen to correspond with the durations of aquatic toxicity studies of indicator aquatic species. As described in Table 4, the durations chosen for comparing predicted concentration with ecotoxicologically relevant concentrations were 24-hour (peak) concentrations for invertebrate species and 96 hours (4 days) for both fish and plant species.

As shown in Table 4 none of the exposure ratios exceed 1. This is not surprising as each of the pesticides is currently registered for use in turf and its use would presumably have been restricted by US EPA if predicted concentrations exceeded a level known to harm a target species. The pesticides are color coded in Table 4 based on the value of their exposure ratios relative to glyphosate, which is colored blue. The seven pesticides with ratios all less than or equal to those of glyphosate are colored green. Displacement of these products with glyphosate is not predicted to result in a net benefit to water quality on golf courses. However, the seven other pesticides have at least one exposure ratio greater than those predicted for glyphosate. Of the seven pesticides with higher exposure ratios, five were listed in Table 1 as having a high potential to have their use significantly displaced by the adoption of RRCB. As the increased use of glyphosate made possible by RRCB is not predicted to itself result in any exposure ratios approaching unity (highest value 0.01), one may conclude that any impact of this suite of golf course chemicals on aquatic organisms should be lessened when switching to the transgenic variety.

These predictions result directly from two assumed changes in pesticide use patterns that will flow from the use of RRCB on golf courses. Herbicide use patterns will be reduced by the use of RRCB. It is common to use a preemergence grass herbicide, a postemergence broadleaf herbicide, and a postemergence grass herbicide during the same growing season. RRCB will permit the use of Roundup to control each of these different weed types or timings.

Secondly, many golf turf managers spend considerable effort trying to either eliminate annual bluegrass from turf or managing it as a turfgrass. Strategies to eliminate annual bluegrass tend to be very chemically intensive, and the use of RRCB will negate all of these applications. Annual bluegrass is a unique weed problem in that it is so competitive, it can become a *de facto* turfgrass, requiring active management to prevent it from dying and causing poor turf quality. Fungicide and insecticide applications are made to control disease and insect pests of annual bluegrass, in some cases these applications would be eliminated by the conversion to RRCB since some of these pathogens will not attack creeping bentgrass. Examples of pests that attack annual

bluegrass but not creeping bentgrass include the annual bluegrass weevil and summer patch (*Magnaporthe poae*).

Elimination of annual bluegrass may also reduce pesticide applications in a subtler manner. Diseases are the major pest problem on golf course turf. Annual bluegrass is very disease susceptible, and may be more susceptible to some diseases than creeping bentgrass. If one species or cultivar in a stand is more disease susceptible than other species or cultivars, then the amount of disease in the less susceptible species may be increased since it is growing so closely to a source of inoculum.[17]

4. CONCLUSIONS

The model results indicate that the displacement of several pesticides made possible by genetic modification of creeping bentgrass would reduce the risk of exceeding ecologically toxic pesticide concentrations in water bodies adjacent to golf courses. The exact magnitude of the water quality improvement is largely dependent on the number of golf courses which adopt the new variety.

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Table 1 Physical and chemical properties for all sixteen pesticides included in the simulation modeling

Compound	Brief Description of Typical Use Pattern	Likely Displacement	DT50 (days)	KOC (L/kg)	Molecular Weight (g/mol)	Water Solubility (mg/L)	Vapor Pressure (mPa)	Henry's Law Constant (dimensionless)	pKa	Hydrolysis Halflife (d)	Aqueous Photolysis Halflife (d)	Soil Aerobic Metabolism Halflife (d)	Soil Anerobic Metabolism Halflife (d)
2,4 D	Widely used broadleaf herbicide. Amine salts of readily disassociate. Rarely applied alone, but is typically a component in a 2 or 3-way blend of postemergence herbicides. Bentgrass is readily injured by 2,4-D; a reduced rate of 0.25 kg ha-1 is typically prescribed for bentgrass.	High	14	48	221.04	890	8E-08	1.07E-09	2.8	30	0.053		
Bensulide	Frequently used for preemergence crabgrass or annual bluegrass control because of its excellent safety on creeping bentgrass including use on putting greens.	High	120	3900	397.54	5.6	8E-07	2.87E-06		0.0032	None	5	
Clopyralid	Used for the control of annual and perennial broadleaf weeds in established turf. Provides outstanding control of white clover (Trifolium repens L.) and is the herbicide of choice for this weed that is well adapted and frequently occurs on the low mowing heights found on golf course greens, tees, and fairways.	High	13	36	192	9000	1.7	1.32E-07	2.3	stable	stable		
Dicamba	Used for the control of annual and perennial broadleaf weeds in established turf. A key component of herbicide mixtures, but is rarely, if ever, used alone on turfgrasses. Very mobile and more persistent than 2,4-D.	High	16	13	221.04	8310	1.66	1.81E-08	1.9	stable	0.018	38	
Dithiopyr	Used for the preemergence control of annual grasses, especially crabgrass, and certain broadleaf weeds in established turf. Has reasonable turf safety, but can cause significant turf injury to colonial bentgrass and to certain creeping bentgrass cultivars.	Low	17	1688	401.4	1	0.533	8.64E-05		Stable	17		490
Ethofumesate	Used for the postemergence control of annual bluegrass in established turf. In order to be effective it is applied 3-4 times per growing season, typically in the fall. Has very marginal turf safety and can cause significant injury to Kentucky bluegrass, creeping bentgrass, or perennial ryegrass. It is currently the only herbicide that can be used for the postemergence control of annual bluegrass in cool-season	High	80	276	286.3	110	0.65	1.49E-06		0.00034	0.533	143	759

Compound	Brief Description of Typical Use Pattern	Likely Displacement	DT50 (days)	KOC (L/kg)	Molecular Weight (g/mol)	Water Solubility (mg/L)	Vapor Pressure (mPa)	Henry's Law Constant (dimensionless)	pKa	Hydrolysis Halflife (d)	Aqueous Photolysis Halflife (d)	Soil Aerobic Metabolism Halflife (d)	Soil Anerobic Metabolism Halflife (d)
	turfgrass.												
Fenoxaprop-p-ethyl	Used for the postemergence control of annual grasses in established turf. Has very marginal safety on creeping bentgrass, and is often applied sequentially at low rates on three week spacing to minimize the potential injury to creeping bentgrass.	Low	12	9490	361.8	0.9	0.004	6.46E-07		0.001	0.097		30
Glyphosate	Extremely broad-spectrum postemergent control of annual and perennial plants. No preemergent (soil) activity.	N/A	32	9049	169.09	12000	6.77E-08	7.93E-13	5.6	Stable	Stable	14	
Imidacloprid	A chloronicotinyl insecticide that has low mammalian toxicity, and is used for the control of insect pests in turf. The insecticide of choice to control the annual bluegrass weevil.	Low	190	411	255.7	510	1.5E-09						
MCPP	Used for the postemergence control of annual and perennial broadleaf weeds in established turf. Has excellent safety on creeping bentgrass and can be used alone for weed control in bentgrass putting greens and tees. More commonly, mecoprop is used in 2- and 3-way mixtures with herbicides such as 2,4-D and dicamba.	High	21	9	214.65	734	0.31	7.44E-07	3.11	stable			
Oxadiazon	Used for the preemergence control of annual grasses in established turf. Provides effective control of goosegrass (Eleusine indicaL.) an annual grass that becomes more troublesome than crabgrass in warmer climates.	High	75	3445	345.23	0.7	0.015	2.91E-06		Stable	25		180
Paclobut-razole	Used as a growth regulator in turf. It is widely used because it also provides annual bluegrass control. Use of paclobutrazol permits a gradual transition to the predominantly creeping bentgrass turf desired by turf managers.	Low	200	500	293.8	26	0.001	4.56E-09		stable	stable		
Pendimethalin	Used for the preemergence control of annual grass weeds in established turf. Widely used in general turf weed control, however, it is fairly injurious to creeping bentgrass and is not labeled for use on creeping bentgrass greens and tees.	High	174	13400	281.3	0.275	1.2	4.96E-04		Stable			60
Prodiamine	Used for the preemergence control of annual grass and broadleaf weeds in established turf. Prodiamine is not labeled for use on putting green turf, but is	High	120	13000	350.3	0.013	0.0033	3.59E-05	no	Stable	20		

Compound	Brief Description of Typical Use Pattern	Likely Displacement	DT50 (days)	KOC (L/kg)	Molecular Weight (g/mol)	Water Solubility (mg/L)	Vapor Pressure (mPa)	Henry's Law Constant (dimensionless)	pKa	Hydrolysis Halflife (d)	Aqueous Photolysis Halflife (d)	Soil Aerobic Metabolism Halflife (d)	Soil Anerobic Metabolism Halflife (d)
Propiconazole	labeled for all other turf uses. A broad spectrum, systemic fungicide that provides disease control in turf. Widley used for the control of soil-borne patch diseases that are particularly difficult to control. Very effective against summer patch, *Magnaporthe Poae*, a disease of annual bluegrass.	Low	115	648	342.2	110	0.056	7.75E-08	1.09	Stable	0.0028	53	84

Table 2. Actual irrigation amounts (cm) added during the 36-year simulation (irrigation rule: add 80% of net ET loss on any day when natural rainfall was less than simulated ET loss)

	May	June	July	August	September
Average	1.93	3.84	3.78	3.66	1.52
Maximum	3.10	6.50	6.71	5.41	2.90
Minimum	0.05	0.89	0.71	1.12	0.08

Table 3. Pesticide application scenarios included in modeling plan

Pesticide	Application Rate (kg/ha)	Application Dates
2,4 D	1.12	15-May, 15-Sep
Bensulide	11.2	15-Apr, 30-Aug
Clopyralid	0.22	15-May, 15-Sep
Dicamba	1.12	15-May, 15-Sep
Dithiopyr	0.56	15-Apr, 30-Aug
Ethofumesate	0.82	15-Apr, 15-Sep, 15-Oct, 15-Nov
Fenoxaprop-p-ethyl	0.0336	20-Jun, 11-Jul, 1-Aug, 22-Aug
Glyphosate	3.36	1-May, 1-Jul, 1-Sep
Imidacloprid	0.44	1-Jul
MCPP	0.56	15-May, 15-Sep
Oxadiazon	3.36	15-Apr
Paclobutrazole	0.56, 0.82, 0.56	15-Apr, 1-Jun, 15-Jul
Pendimethalin	3.36	15-Apr, 30-Aug
Prodiamine	0.72	15-Apr
Propiconazole	1.98	1-May, 1-Jun

	Estimated Environmental Concentration (EEC) in Standard Farm Pond (mg/L) (10-ha field) [a]					Lowest Acute LC_{50}/EC_{50} Value [b] (mg/L), FW aquatic species			Maximum Exposure Ratio (Exposure/Toxicity Value)		
Pesticide	Peak	96-hour	21-day	60-day	90-day	Invertebrate	Fish	Plant	Invertebrate [c]	Fish [d]	Plants [d]
2,4-D	0.0191	0.0173	0.0123	0.0064	0.0043	25	250	0.58	< 0.01	< 0.01	0.0298
Bensulide	0.0289	0.0039	0.0011	0.0004	0.0003	0.58	0.38	0.14	0.050	0.0103	0.028
Clopyralid	0.0100	0.0099	0.0096	0.0092	0.0084	750	103.5		< 0.01	< 0.01	
Dicamba	0.0019	0.0002	0.00005	0.00002	0.00001	> 1.0	28	0.061	< 0.002	< 0.01	< 0.01
Dithiopyr	0.0024	0.0022	0.0019	0.0016	0.0013	0.58	0.46	0.02	< 0.01	< 0.01	0.112
Ethofumesate	0.027	0.027	0.026	0.0219	0.0173	5.3	0.75	> 2.76	< 0.01	0.036	< 0.01
Fenoxaprop	0.00005	0.00004	0.00002	0.00001	0.00001	0.098	0.31	0.43	< 0.01	< 0.01	< 0.01
Glyphosate	0.0081	0.0069	0.0058	0.0055	0.0054	55	22	0.64	< 0.01	< 0.01	0.0108
Imidacloprid	0.0069	0.0068	0.0065	0.0061	0.0049	85.2	> 83		< 0.01	< 0.0001	
MCPP (mecoprop)	0.0094	0.0012	0.00023	0.00008	0.00005	> 100	> 92	0.24	< 0.	< 0.	< 0.01
Oxadiazon	0.0048	0.0026	0.0012	0.0007	0.0005	0.27	0.88	0.078	0.0176	< 0.01	0.033
Paclobutrazol	0.029	0.028	0.027	0.022	0.021	33.2	23.6	33.5	< 0.01	< 0.01	< 0.01
Pendimethalin	0.0051	0.0035	0.0020	0.0017	0.0016	0.28	0.138	0.0054	0.018	0.026	0.654
Prodiamine	0.0004	0.0002	0.0001	0.00004	0.00003	> 0.083	> 0.55		< 0.005	< 0.0003	
Propiconazole	0.043	0.042	0.040	0.034	0.032	0.51	0.83	0.093	0.0837	0.0508	0.4531

[a] 90th percentile EEC; EEC predicted to be exceeded 1 year out of every 10 years.
[b] Value for freshwater species from U.S. EPA (2000) unless otherwise stated below.
[c] Peak EEC values used to calculate exposure ratio.
[d] 96-hr EEC values used to calculate exposure ratio.
Glyphosate data taken from Giesy et al. (2000).
Clopyralid invertebrate data: EPA ECOTOX Database.
Clopyralid fish data: Hazardous Substance Data Bank (HSDB).
Paclobutrazol algal data: EPA ECOTOX database.
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Appendix III

Literature Review of the *Agrostis* spp. and related genera of North America

Personal Communication

Bruce MacBryde

USDA APHIS BRS

Appendix IV

Reports from the literature citing putative and confirmed hybrids between *A. Stolonifera* and other related species and genera

Latin Name	Synonyms	Common Name	2n	Adaptation	Cross Frequency or ratio	Confirmed Hybrid Reference	Other Reference. Putative + Fail	Barriers to crossing and persistence
Agrostis stolonifera L.	*A. palustris*	Creeping bentgrass	28	Marshy, fertile lowlands				Distance, pollen competition.
A. capillaris	*A. tenuis* Sibth. *A. vulgaris*	Colonial bentgrass Browntop	28	Variable Davies, 1953.	NA	Davies, 1953	Jones, 1956 examines meiosis of Davies hybrids. Bradshaw, 1958 examines morph. of Davies hybrids and field collection.	Anthesis time of parents Davies, 1953. Philipson, 1937 First anthers of hybrid fail to dehisce. Davies, 1953 Meiotic irregularity. Jones, 1953., 1956. Low fertility of hybrids. Davies, 1953; Bradshaw, 1958. "Limited power of dispersal." Bradshaw, 1958.
					NA	Wipff, Pure Seed Testing, 1999 Wipff and Fricker, 2001		
					0.1 / panicle	Belanger, 2000		
					0.00044	Belanger, 2001		
							Fouillade, 1932 Putative	
							Hegi, 1935. Putative	
A. castellana Bois. et Reut.		Dryland bentgrass 'Highland'	42		0.07 / panicle	Belanger, 2000		Anthesis date (Faith Belanger, 2000) Chromosome number
					0.000015	Belanger, 2001, 2002		
					NA	Wipff, Pure Seed Testing, 1999, Wipff and Fricker, 2001		
A. alba L.	*A. gigantea* Roth.	Redtop	42	Damp shaded and arable land.	0		Davies, 1953 Failed forced cross	Anthesis date (Belanger, 2000) Anthesis time Davies, 1953. Philipson, 1937 Meiotic irregularity. Poor fitness of aneuploid hybrids makes field survival unlikely. Jones, 1956.b
					0.4 seed / panicle	Belanger, 2000		Anthesis date, cross incompatibility
					NA	Wipff, Pure Seed Testing, 1999		

Latin Name	Synonyms	Common Name	2n	Adaptation	Cross Frequency or ratio	Confirmed Hybrid Reference	Other Reference. Putative + Fail	Barriers to crossing and persistence
						Wipff and Fricker, 2001		
					0.000000		Belanger, 2001, 2002	Anthesis nick date and time of day
A. canina var. *fascicularis*	*A. canina canina*	Velvet bentgrass	14	Wet damp soils Davies, 1953.			Davies, 1953	Anthesis time Davies, 1953 Philipson, 1937. Low fertility of hybrids with other *Agrostis* Davies, 1953.
							Clapham, et al 1952 Putative ref.	
							Philipson, 1937 Putative ref.	
					0.046 seed / panicle	Belanger, 2000		Anthesis date, incompatibility
					NA	Wipff, Pure Seed Testing, 1999 Wipff and Fricker, 2001		
					0.000		Belanger, 2001, 2002	Anthesis date and time of day
A. canina var. *arida*	*A. vinealis* Schreber. *A. canina montana* Hartm. *A. trinnii*	Brown bent	28				Davies, 1953	Anthesis time of day. Davies, 1953 Meiotic irregularity of hybrids. Jones, 1953. Sterility of hybrids. Jones, 1956.
A. canina							Philipson, 1937	
A. exarata	*A. ampla, A. longiligula*	Spike bentgrass	56	Native Wetland Water limits colonization in West. Lamson Scribner, 1897	NA		Carlbom, 1967 Putative Welsh et al, 1987 Putative	Cleistogamous. Carlbom, 1967. Chromosome # 42 and 56.
A. pallens		Dune bent Seashore bent	42	Coastal	5/57	Wipff and Fricker, 2001		Coastal endemic
A. scabra:		Rough bent	42	Med. to high elevation	NA		Welsh et al., 1987 Putative	Allogamous

Latin Name	Synonyms	Common Name	2n	Adaptation	Cross Frequency or ratio	Confirmed Hybrid Reference	Other Reference. Putative + Fail	Barriers to crossing and persistence
Polypogon monspeliensis		Annual rabbitsfoot grass		Wet ground	NA		Camus, 1958	
P. fugax	*A. stolonifera X P. monspeliensis* *A littoralis* *P littoralis*			Wet ground	NA		Hubbard, applies name for putative hybrids of *A. stolonifera* with *P. monspeliensis*	
P. littoralis	*P. fugax* *A littoralis,* *A. stolonifera X* *P. monspeliensis*			Wet ground	NA		Philipson, 1937 reports herbarium specimen appears to be a hybrid of *A. stolonifera* and *P. monspeliensis* .	
P. semiverticillata	*P. viridis* *A verticillata* *A semiverticillata* (Forsk.)	Water bent	42	Wet ground	NA		Welsh et al., 1987 reports putative hybrid sighting.	
P. viridis	*A verticillata* *A semiverticillata* (Forsk.) P semiverticillata	Water bent	42	Wet ground	NA			
P. elongatus				Wet ground	NA		Parodi, 1951 Putative	

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Appendix V

Plant characteristics of transgenic hybrids formed between Roundup Ready® Creeping Bentgrass and related *Agrostis* and *Polypogon* species.

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and Peter Raymond, Monsanto Company

Plant characteristics of transgenic hybrids formed between Roundup Ready® Creeping Bentgrass and related *Agrostis* and *Polypogon* species.

Jim Frelich, Eric Nelson, and Peter Raymond

ABSTRACT

Creeping bentgrass (*Agrostis stolonifera* L.) is a perennial, stoloniferous, wind-pollinated, obligate outcrossing grass species. As such, sexually receptive females are pollinated and fertilized by wind borne pollen from compatible, neighboring and adjacent plants. Creeping bentgrass can also potentially outcross with sexually receptive plants of related species (Davies, 1953; Wipff and Fricker, 2001; Belanger *et al.*, 2003; Bjorkman, 1954; Welsh *et al.* 1987; Tutin, 1980). Pollen-mediated transgene flow has been raised as a potential impact of product release and is addressed by regulators prior to de-regulation (USDA, CFIA)(Kohler, 1997)

Pollen movement and pollen-mediated gene flow is not unique to transgenic plants. Within the turfgrass industry, identity preserved programs including variety descriptions and established tolerances for genetic purity have been successfully addressed. Rules for Certified eligible varieties that address appropriate labeling, field history for planting and isolation distance between varieties of the same and cross-compatible species have been established.

One of the ecological concerns about genetically engineered crop plants focuses on the potential for transgenic crop plants to become weeds in agriculture or transmit the inserted trait into related species resulting in a selective advantage to the receiving species. Further, that those species might then become invasive of natural habitats or pose a threat to agriculture beyond what may already occur for existing conventional species.

Herein, we describe a study that examined the plant vegetative characteristics of recovered transgenic hybrids between Roundup Ready®[1] Creeping Bentgrass event ASR368 and related species formed in the field in Franklin County, WA (Christoffer 2003). Direct comparisons of the performance of recovered hybrids were made in 2001 and 2002 to representative populations of the maternal and paternal parent species. Plants of the parent species were grown from seed samples of commercial cultivars naturalized populations and/or plant introductions (PI) from stations in Washington and Georgia that represent the Germplasm Resources Information Network (GRIN). Seed harvested from related maternal species (including *Agrostis capillaris*, *A. gigantea*, *A. idahoensis*, *A. pallens*, *A. trinii*, *Polypogon monspeliensis*, *P. fugax* and *P. viridis*) and hybrid event ASR368 seeds were germinated in the greenhouse at the same time as seed of the parental species. Roundup tolerant hybrid seedlings were identified with repeat applications of Roundup herbicide. Sixty (where possible) random hybrid seedlings from

[1] Roundup and Roundup Ready are registered trademarks of Monsanto LLC.

several representative plants of each species and the parent species were transplanted to individual pots prior to estimation of ground coverage, growth habit and tiller density.

The comparisons between hybrids and the parent species in both 2001 and 2002 showed that the recovered event ASR368 hybrids were no more competitive than the populations representing the parent species. None of the recovered event ASR368 hybrids displayed higher rates of vegetative growth, or changes in plant growth habit when compared to the parent species. Therefore, it is concluded that interspecific and intergeneric hybridization with event ASR368 confers no novel growth characteristics that would make hybrid plants any more invasive than the respective parent species.

INTRODUCTION

Background

The Monsanto Company and The Scotts Company have collaborated to develop a Roundup Ready®[1] variety of creeping bentgrass (*Agrostis stolonifera* L. syn. *Agrostis palustris* Huds., syn. *A. stolonifera* var. *palustris* (Hudson) Farw. (Philipson, 1937; Soreng *et al.*, 2001). A plasmid containing the *ctp2: cp4 epsps* gene expression cassette was transferred to creeping bentgrass via the particle bombardment method. This same gene has been used in the production of Roundup Ready corn, soybeans, sugar beet, and canola .

Creeping bentgrass is a perennial, stoloniferous, cool season grass predominantly used for recreational turf on golf course greens, tees, and fairways in cool season turfgrass growing areas of the United States (Beard, 2002). The species is best adapted to wet, low-lying, damp, marshy areas in coastal regions and inland along ditches and similarly wet areas with a neutral pH (Bradshaw, 1958a,b; Hitchcock, 1950, Hanson, et al., 1969). Creeping bentgrass is well adapted to golf courses because of its low growing, creeping habit that allow it to tolerate frequent close mowing, foot and equipment traffic and to recuperate from wear and divots while providing an aesthetically pleasing and uniform playing surface along with all of the other benefits attributable to turfgrasses. The high maintenance requirements for creeping bentgrass including frequent (3-7 times / week) close (3-18mm) mowing, consistent plant-available soil moisture and nutrients, and pest control essentially limits creeping bentgrass use to the golf market.

One of the ecological concerns about genetically engineered crop plants focuses on the potential for transgenic crop plants to become weeds or hybridize with other species that may become weeds in agriculture or natural habitats. Factors associated with the recovery of hybrids between creeping bentgrass and related species are sexual compatibility, flower synchrony, prevailing wind direction, pollen movement, pollen viability, and distance from pollen source. Relative frequency of pollen-mediated interspecific gene flow from paternal *A. stolonifera* plants containing the *bar* gene (used as a selectable marker that confers resistance to the herbicide glufosinate ammonium) to 4 closely related *Agrostis* species up to 15 m away from the transgene donor has been studied. The species examined and the mean frequency of hybrid recovery were: *A.*

tenuis (0.00044), *A. castellana* (0.000015), *A. gigantea* (0.00000), *A. canina* (0.000). (Belanger *et al.*, 2003).

Recent field results have expanded the knowledge base on interspecific and intergeneric hybridization as influenced by species, prevailing winds, and distance from the transgenic pollen source (Christoffer, 2003). Outcrossing potential of Roundup Ready Creeping Bentgrass (RRCB), as measured by the frequency of recovered, glyphosate-tolerant progeny, was greatest with transgene (*cp4 epsps*) flow from RRCB to pollen receptor *Agrostis stolonifera*. Other interspecific and intergeneric, glyphosate-tolerant hybrids were also recovered from related *Agrostis* and *Polypogon* species, but frequencies were much lower than for intraspecific crosses at the closest distance to the transgenic pollen source.

Pollen movement and pollen-mediated gene flow are not unique to transgenic plants. Within the turfgrass industry, identity preserved programs including variety descriptions, and established thresholds for genetic purity have been successfully addressed with rules for Certified varieties that require appropriate labeling, field history for planting, and isolation distance between varieties of the same and cross-compatible species. These rules are developed by the Association of Official Seed Certifying Agencies (AOSCA, 2001) and by state seed certification agencies such as the Oregon Seed Certification Service (Oregon Seed Certification Manual, 2001). Seed growers alone are responsible for implementing appropriate strategies for protecting the genetic purity and integrity of their crop fields from the date of planting through harvest so that the seed will pass the rigid standards necessary for Certified status. State seed certifying agencies typically inspect and enforce the standards for the production and sale of pedigree seed within their state (Oregon Seed Certification Handbook, 2001). These grass seed certification programs have been in place and successfully implemented for over 40 years.

Interspecific hybridization in *Agrostis* has been examined since at least the 1950's (Davies, 1953; Philipson, 1937; Bjorkman; 1960). However, the difficulty in diagnosis of independent species through morphological and adaptive characters that overlap considerably from one species to another, calls into question the extent of true species delineation within the genera (Philipson, 1937). Examinations of *Agrostis* hybridization were initiated in Great Britain by Davies (1953), Jones (1956a,b,c) and Bradshaw (1958 a,b). These researchers attempted to address whether the overlap in characters observed among different species within the *Agrostis* genus resulted from interspecific hybridization. Forced crosses and cytological examination of meiotic pairing in gametes confirmed the hybrid nature of Davies' and Jones' plants. Bradshaw demonstrated that characteristics of the hybrids including biomass, stolon length, shoots per unit area and plant spread were generally equal to one of the parents or intermediate to both of the parents. The interspecific hybrids studied by Jones have however been a valuable source of information on the taxonomy and genetics of the genus. Hybrids may serve to provide additional genetic information as biotechnology progresses in the grass family.

Reports of putative intergeneric hybridization between *Agrostis* and *Polypogon* genera are rare, but present in the literature (Welsh, 1987; Bjorkman, 1960; Camus, A. 1958.

Parodi, 1951; Philipson, 1937). However, true hybrid status is most often unsubstantiated. Reports of any cytological examination of putative intergeneric hybrids used to confirm hybrid status have not been found in the literature. In addition, the taxonomic confusion over placement of plants in the *Agrostis* genus or *Polypogon* genus is apparent in the literature (Hitchcock, 1950; Carnahan and Hill, 1961; USDA, NRCS. 2001.)

No reports documenting agricultural or ecological impact associated with interspecific or intergeneric *Agrostis* hybrids beyond that of their parent species have been found in the literature.

Herein we describe a study that examined plant vegetative characteristics of recovered transgenic hybrids between Roundup Ready®[2] Creeping Bentgrass and related species formed in the field (Christoffer, 2003). Direct comparisons of the performance of recovered hybrids were made to a number of plant collections in 2001 and 2002. Plants of the parent species were grown from seed samples of commercial cultivars, naturalized populations and/or plant introductions (PI) from stations in Washington and Georgia representing the Germplasm Resources Information Network (GRIN). Seed harvested from related maternal species (including *Agrostis capillaris*, *A. gigantea*, *A. idahoensis*, *A. pallens*, *A. trinii*, *Polypogon monspeliensis*, *P. fugax* and *P. viridis*) and hybrid event ASR368 seeds were germinated in the greenhouse at the same time as seed of the parental species.

Comparative evaluation of Roundup ready event ASR368 to commercial creeping bentgrass cultivars representative of *A. stolonifera* revealed no selective advantage conferred by the introduced *cp4 epsps* gene and protein. Thus, it would be expected that no advantage would be conferred to a hybrid formed between ASR368 and a related species with which it can interbreed. However, APHIS requested confirmatory data on hybrid development that is presented here.

MATERALS AND METHODS

Plant materials used in this study include multiple recovered transgenic hybrids between Roundup Ready® creeping bentgrass event ASR368 and related species and their respective parent (conventional) plant populations. Plants of the parent species were grown from seed samples of commercial cultivars, naturalized populations and/or plant introductions (PI) from stations in Washington and Georgia representing the Germplasm Resources Information Network (GRIN).

The source of all transgenic plants (glyphosate-tolerant hybrids) used in this experiment were generated by Christopher (2003) in the "*Agrostis stolonifera* L. Pollen-Mediated Intraspecific, Interspecific and Intergeneric Transgene Flow" study conducted in Washington in 2001.

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[2] Roundup and Roundup Ready are registered trademarks of Monsanto LLC.

Each of the hybrids evaluated met and satisfied the Christopher (2003) RR progeny criteria that involved two Roundup Pro® spray applications (Table 1). All non-transgenic (conventional) parent plant material is listed on Table 1. Several parent plant species were not represented by a recovered hybrid.

Christopher (2003) harvested seed from eight maternal species during the summer of 2001. The transgenic and the non-transgenic parents were seeded into sand flats on August 24, 2001 and germinated in a polyhouse. The sixty- (60) glyphosate tolerant progeny (hybrids) seedlings were recovered from several representative plants of each species by two Roundup Pro spray applications, one 9 days after emergence and the second on September 21, 2001. Roundup Pro herbicide was applied at 3.5 kg ae/ha using a tank mixture of 50% Roundup Pro® and 50% Accord® to all flats. The herbicide was applied with a hand held CO2 pressurized spray unit with an output of 374 L/ha (1st application) and 187 L/ha (2nd application) using 1105 TeeJet nozzles at 221 kPa. The non-transgenic (conventional) parent plants were not treated with Roundup Pro. The transgenic hybrids and the non-transgenic parent species seedlings were removed from the sand flats and transplanted into Jiffy® Pellets (42 mm) on October 15-19, 2001 in Marion County, Oregon.

Experiment I - Greenhouse

For experiment I, plant/pellets were transplanted into 14 cm pots containing a peat/perlite (95:5, V:V) growing media (10 cm deep) on November 22-24, 2001. All containerized plants were placed and maintained in an unheated cold frame on a graveled site where temperature remains similar to ambient temperature. Each plant received an application of fertilizer with analysis of 27-3-4-2 at a rate of 1.0 lb. Nitrogen/M rate on January 18, 2002. The plants were then moved to a cold-framed poly house on February 7, 2002 to induce early spring green up. Each plant received water via irrigation system to prevent moisture stress. The ambient environment in the poly house was not controlled except for open doors during the day to control humidity and closed at night. Daytime high temperatures ranged between 13-19°C and nighttime lows between 5-10°C. No fungicides were used on the plants. The experimental design was a randomized complete block with three replications. Each transgenic hybrid was represented by up to 20 plants with subsamples of each hybrid population derived from up to 4 different maternal plants from each species per block (Table 1 and Appendix Table A1). One plot consisted from three up to seven plants (subsamples) each depending upon plant count availability of each hybrid source.

Data collections included three parameters: 1) percent plant cover, 2) tiller capacity and 3) plant type. Vegetative spring growth was estimated as percent cover of each container by the plant represented by the parental species collections or the recovered hybrids on February 20, 2002. Tiller capacity per plant was estimated using a scale of 1 to 5 where, 1 = 1-5 tiller, 2 = 6-20 tillers, 3 = 21-50 tillers, 4 = 51-90 tillers and 5 = greater than 91 tillers. An average for each population was then calculated prior to analysis in Agricultural Research Manager (ARM). Plant type was also estimated using a categorical 1 to 3 scale where 1 = bunch, tufted, erect, non-spreading, 2 = pseudo erect,

not strongly bunched, tufted, nor decumbent, and 3 = spreading, decumbent, prostrate. All observations were made 91 days after transplanting into the containers (or 180 days after seeding).

Experiment II - field

Vegetative tillers of the transgenic hybrids and non-transgenic parent species were removed from containerized (14 cm pots) plants growing in the peat/perlite media, and sprigged into Jiffy Pellets (42mm x 10 cm deep) on June 10, 2002. All plantlets were maintained on a graveled site under ambient temperature. Irrigation was provided daily as needed until movement into the field in the fall 2002. Each plant received an application of 27-3-3 fertilizer at a rate of 1.0 lbs N/M on June 18, 2002 and August 5, 2002.

The plantlets were moved and transplanted in the field on August 15 and 16, 2002 in Marion County, Oregon. The planting occurred into cultivated and cultivator-packed Willamette silt loam soil. Holes were hand dug, plant/pellets were placed in the hole and covered with ½ inch of soil to prevent exposure of the surface plantlets to air. Plant spacing was on 1.5 ft centers and rows were spaced 3 ft apart. Soil fertility and moisture was maximized to promote an optimum growing environment for all plants during the duration of the study. Once transplanting was completed, an application of 46-0-0 fertilizer was made at a rate of 1 lbs N/M rate on August 17, August 23, and September 13, 2002 and watered with 1.5 inches of irrigation. Irrigation was then provided to prevent any sign of plant stress. All non-target weeds were controlled manually on September 16 and 17, 2002. The test was set up in a randomized complete block design, with four replications. Each transgenic hybrid was represented from up to 12 plants with 3 sub samples of each hybrid genotype for each species group (Table 2 and Appendix Table A1).

Plant growth and spread were recorded on October 13, 2002. The diameter of each plant canopy was recorded in centimeters as a measurement of plant growth and spread. The plant diameter data was transformed into ground cover density based on the field space provided per individual plant. All observations were made two months after planting. The non-transgenic parent species were used as reference comparisons for the transgenic hybrids.

Table 1. Conventional (non-transgenic) parent species used as reference comparisons for the recovered transgenic hybrids of the same parent species in Experiment I in Marion County, Oregon.

Scientific Name	Common Name	Plant Type Transgenic or Conventional*
Agrostis stolonifera L.	Creeping Bentgrass	C
Agrostis capillaris L.	Colonial Bentgrass	C, H
Agrostis gigantea Roth.	Redtop Bentgrass	C, H
Agrostis idahoensis Nash.	Idaho Bentgrsss	C, H
Agrostis pallens	Dunes Bentgrass	C, H
Agrostis trinii Turcz.	Brown Bentgrass	C, H
Polypogon monspeliensis L. Desf.	Rabbitsfootgrass	C, H
Polypogon viridis (Gouan) Breistr.	Watergrass	C, H
Polypogon fugax Nees es Steud.		C, H
Agrostis castellana Boiss.& Reut.	Dryland Bentgrass	C,
Agrostis cania L.	Velvet Bentgrass	C,
Agrostis vinealis Schreb.	Brown Bentgrass	C,
Agrostis sp.	Rhode Island Bent	C,

* Where C = conventional and H = hybrid formed between RRCB event ASR368 and conventional

Table 2. Conventional (non-transgenic) parent species used as reference comparisons for the recovered transgenic hybrids of the same parent species in Experiment II in Marion County, Oregon.

Scientific Name	Common Name	Plant Type Transgenic or Conventional*
Agrostis stolonifera. L.	Creeping Bentgrass	C,
Agrostis capillaries L.	Colonial Bentgrass	C, H
Agrostis gigantea Roth.	Redtop Bentgrass	C, H,
Agrostis idahoensis	Idaho Bentgrass	C, H
Agrostis pallens	Dunes Bentgrass	C, H
Agrostis pallida		H
Agrostis trinii		H
Polypogon monspeliensis L. Desf.	Rabbifootgrass	C, H
Polypogon viridis (Gouan) Breistr.	Watergrass	C, H
Polypogon fugax Nees es Steud		C, H

* Where C = conventional and H = hybrid formed between RRCB event ASR368 and conventional

RESULTS AND DISCUSSION

Experiment I

Significant differences were observed between species and also between parent species plant collections and recovered glyphosate-tolerant hybrids in Experiment I (Table 3). The recovered hybrids were either not significantly different from their respective maternal and paternal parent species collections or had considerably less vegetative growth than their respective parental species plants.

Recovered, glyphosate-tolerant hybrids from *A. trinii* x *A. stolonifera* were less vigorous than the non-transgenic *A. trinii* parent species plant collections. A larger percentage of the hybrid plants were observed with fewer, more erect tillers than the representative *A. trinii* parent species plants.

Reduced growth was observed among recovered hybrids from *A. idahoensis* x *A. stolonifera* when compared to representative non-transgenic *A. idahoensis* parent species plant collections. Hybrid plants exhibited a more erect growth habit, but hybrids in general had fewer tillers per plant.

The growth rate of recovered hybrids from *A. pallens* x *A. stolonifera* was also significantly less than its respective parent non-transgenic *A. pallens* species plants with many of the hybrid plants having a weaker tillering capacity and more erect growth habit. No meaningful biological differences were seen between the recovered hybrids from *A. capillaris* x *A. stolonifera* when compared to its representative parent species plants.

The vegetative growth rate as measured by percent ground cover of the recovered hybrids for *A. gigantea* x *A. stolonifera* were seen as significantly higher than for the respective *A. gigantea* parent species plants but not significantly different from *A. stolonifera*. Plants from the four hybrid subsamples were similar in tiller capacity and plant growth habit to the respective parent species populations.

Polypogon monspeliensis x *A. stolonifera* parent species plants and the recovered glyphosate-tolerant hybrids recovered from *P. monspeliensis* were not different with respect to growth rate as measured by ground cover. There was a slight shift toward plants having slightly more tillers per plant among hybrids than among the *P. monspeliensis* parent plants but they generally did not differ in number of tillers per plant compared to the *A. stolonifera* parent plants. A comparison between *P. fugax* parent species plants and the recovered hybrids showed no apparent differences in growth rate, tillering capacity and growth habit. Recovered, event ASR368 hybrids from *P. viridis* were seen as less vigorous. A larger percentage of plants with a higher percentage of tillers exhibiting a more erect growth habit than representative parent species collections from *P. viridis* or *A. stolonifera* were observed.

Experiment II

All of the recovered glyphosate-tolerant hybrids produced considerably less vegetative growth and spread when compared to the reference (non-transgenic) *A. stolonifera* parent species. The recovered hybrids were either non-significantly different from or smaller in vegetative growth and spread when compared to the related non-transgenic parent species (Table 4).

Vegetative spread as measured by plant diameter of the recovered hybrids of *A. idahoensis* and *A. trinii* were observed as slightly greater but not significantly different from their non-transgenic *A. idahoensis* and *A. trinii* parents, respectively.

CONCLUSIONS

The potential of a plant to become an established weed in managed agricultural systems generally depends upon several plant characteristics. Many genes working in combination to acquire a specific adaptive trait or traits that provide it with enhanced tolerance or increased survival in a specific niche are required. A single gene such as that which confers Roundup tolerance is unlikely to impart any additional competitive advantage in unmanaged ecosystems where Roundup is not normally applied (Quemada, 1999). In addition, as demonstrated in this study, it is unlikely that the insertion of the Roundup Ready trait will impart any competitive advantage in managed ecosystems where Roundup is not the only herbicide used. There are many herbicides commonly used in turf and grass seed production settings that elicit phytotoxic reactions in bentgrass and elicit no, or lesser, phytotoxic reaction in other grasses such as bluegrasses, ryegrasses and tall fescue (Bingham, 1989). In addition, tiny bentgrass seeds (6-7,000,000 per pound) and seedlings are poor invaders of established plant stands (Howe and Snaydon, 1986; Rossi, 1999). It is unlikely that a low frequency event such as interspecific or intergeneric hybridization would survive establishment in the midst of the plant that set the seed. The results of these two studies demonstrate that even if a hybrid seed were set and a seedling did establish itself somewhere, it is unlikely to be distinguished from its parent species by growth rate, tillering capacity or growth habit or to be any more invasive than plants that exist among the *Agrostis* and *Polypogon* genera today.

Table 3. Plant characteristics of hybrids recovered from event ASR368 and related species in 2001.

Plant Population[1]	Percent Ground Cover[2]	Tillers/Plant (1-5[3])					Growth Habit (1-3[4])		
		% Occurrence per Rating Unit							
		1	2	3	4	5	1	2	3
A. stolonifera (Creeping Bent) Conventional	20.47 d	0	17	67	17	0	44	27	27
A. capillaris (Colonial Bent) Hybrid	14.33 h-k	0	33	67	0	0	67	33	0
A. capillaris (Colonial Bent) Conventional	15.50 e-h	0	33	44	22	0	56	11	33
A. gigantea (Red Top) Hybrid	15.00 f-I	0	50	50	0	0	75	17	8
A. gigantea (Redtop) Conventional	10.65 jkl	22	44	33	0	0	83	17	0
A. idahoensis (Idaho Bent) Hybrid	9.43 L	11	56	22	11	0	67	33	0
A. idahoensis (Idaho Bent) Conventional	14.53 g-j	0	44	44	11	0	22	22	56
A. pallens (Dunes Bent) Hybrid	10.75 jkL	0	58	33	8	0	67	17	17
A. pallens (Dunes Bent) Conventional	29.33 a	0	0	67	33	0	33	67	0
P. monspeliensis (Rabbitsfootgrass) Hybrid	19.43 de	0	0	67	33	0	78	22	0
P. monspeliensis (Rabbitsfootgrass) Conventional	18.30 d-g	22	44	22	11	0	89	11	0
P. fugax Hybrid	20.13 d	0	44	56	0	0	100	0	0
P. fugax Conventional	21.80 cd	0	56	33	11	0	89	11	0
P. viridis (Watergrass) Hybrid	15.23 f-I	0	67	33	0	0	100	0	0
P. viridis (Watergrass) Conventional	25.13 bc	0	67	22	11	0	22	78	0
A. castellana (Dryland Bent Conventional	28.37 ab	6	17	39	39	0	50	50	0
A. canina (Velvet Bent) Conventional	18.57 def	0	22	44	22	11	44	22	33
A. trinii Hybrid	5.33 m	36	64	0	0	0	100	0	0
A. trinii Conventional	10.40 kl	0	89	11	0	0	100	0	0
A. vinealis (Brown Bent) Conventional	10.50 kl	0	44	56	0	0	67	22	11
A. sp.(Rhode Island Bent) Conventional	19.53 d	0	44	44	11	0	67	33	0
LSD (p=0.05%)	3.964								
Stn. Deviation	3.433								

[1] conventional or hybrid formed between RRCB event ASR368 and conventional
[2] Means followed by the same letter are not significantly different (LSD, p = 0.05)
[3] Tillers/plant: 1 = 1 to 5; 2 = 6 to 20; 3 = 21 to 50; 4 = 51 to 90; 5 = greater than 90
[4] Growth habit: 1 = bunch, tufted, erect, not spreading; 2 = pseudo-erect, not strongly bunched, tufted or decumbent; 3 = spreading, decumbent, prostrate

Table 4. Plant characteristics in 2002 of conventional *Agrostis* and *Polypogon* species used as comparators and interspecific and intergeneric RR hybrids with creeping bentgrass event ASR368 recovered from related *Agrostis* and *Polypogon* species in 2001.

Plant Population[1]	Relationship	n	Plant Diameter (cm)[2]	Ground Cover (%)[3,4]
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. capillaris (Colonial Bent) Hybrid	Hybrid	12	33.3fgh	57.1def
A. capillaris (Colonial) Conventional	Parent	12	38.7def	75.5def
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. gigantea (Red Top) Hybrid	Hybrid	16	39.6def	78.6de
A. gigantea (Red Top) Conventional	Parent	20	44.5cde	103.1cd
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. idahoensis (Idaho Bent) Hybrid	Hybrid	12	24.3hi	31.5ef
A. idahoensis (Idaho Bent) Conventional	Parent	12	23.4hi	27.9ef
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. pallens (Dunes Bent) Hybrid	Hybrid	16	36.1efg	63.9def
A. pallens (Dunes Bent) Conventional	Parent	12	39.5def	76.4def
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
P. monspeliensis (Rabbitsfootgrass) Hybrid	Hybrid	12	39.9def	77.5de
P. monspeliensis (Rabbitsfootgrass) Conventional	Parent	12	58.5ab	187.3b
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
P. fugax Hybrid	Hybrid	12	40.1def	84.9de
P. fugax Conventional	Parent	12	67.9a	267.2a
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
P. viridis (Watergrass) Hybrid	Hybrid	12	47.3cd	109.5cd
P. viridis (Watergrass) Conventional	Parent	12	53.2bc	147.bc
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. trinii Hybrid	Hybrid	16	35.9efg	78.7de
A. trinii Conventional	Parent	12	25.3ghi	31.5ef
A.stolonifera (Creeping Bentgrass) Conventional	Parent	24	69.3a	249.2a
A. pallida	Hybrid	16	36.6def	77.8de
LSD (p=0.05%)			11.17	60.44
Std Deviation			7.9	42.74

[1] conventional or hybrid formed between RRCB event ASR368 and conventional
[2] Means followed by same letter are not significantly different (LSD, p = 0.05)
[3] Means followed by same letter are not significantly different (LSD, p = 0.05)
[4] Percent ground cover based on plant spacing of 45.7cm (1.5 ft)

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Table A1. Specific conventional (non-transgenic) parent species used as reference comparisons for the recovered transgenic hybrids of the same parent species use in Experiments I and II.

Entry Number	C or RT	Scientific Name	Common Name	Cultivar, Lot #, or Other I.D. Description
1	RT	*Agrostis capillaries L.*	Colonial Bentgrass	0-0-2-3
2	RT			0-0-2-2
3	RT			0-0-2-1
40	C			'Exeter' (PI # 578528)
41	C			'Astoria' (PI # 578527)
42	C			'SR 7100' (PI # 578228)
4	RT	*Agrostis gigantean Roth.*	Redtop Bentgrass	0-0-5-2
5	RT			0-0-5-2
6	RT			0-0-5-4
7	RT			0-0-5-5
46	C			(PI # 235439)
47	C			(PI # 235439)
48	C			(PI # 440111)
49	C			(PI # 598457)
50	C			(PI # 598457)
8	GT	*Agrostis idahoensis*	Idaho Bentgrass	0-0-6-2
9	GT			0-0-6-4
10	GT			0-0-6-4
43	C			(Lot # 64.86.98-1)
44	C			(Lot # 64.86.98-1)
45	C			(Lot # 64.86.98-1)
11	GT	*Agrostis pallens*	Dunes Bentgrass	0-0-8-3
12	GT			0-0-8-3
13	GT			0-0-8-3
14	C			0-0-8-3
61	C			(PCS Lot # U 3971)
62	C			(PCS Lot # U 3971)
63	C			(PCS Lot # U 3971)
15	GT	*Agrostis pallida*		0-0-10-2
16	GT			0-0-10-2
17	GT			0-0-10-2
18	GT			0-0-10-2
19	GT	*Polypogon monspeliensis (L.) Desf.*	Annual Rabbitfootgrass	0-0-13-1
20	GT			0-0-13-1a
21	GT			0-0-13-1b
31	C			(Lot Madras)
32	C			(PI #602408 01 SD)
33	C			(PI # 380996 01 SD)
22	GT	*Polypogon fugax*	Rabbitfootgrass	0-0-14-5
23	GT			0-0-14-2
24	GT			0-0-14-6
52	C			(PI # 220619)
53	C			(PI # 220619)
54	C			(PI # 220617)

Table A1 (contd.). Specific conventional (non-transgenic) parent species used as reference comparisons for the recovered transgenic hybrids of the same parent species use in Experiments I and II.

25	GT	*Polypogon viridis*	Water Bentgrass	0-0-15-2
26	GT			0-0-15-1
27	GT			0-0-15-3
28	C			(PI #317419 01 SD)
29	C			(PI #287746 01 SD)
30	C			PI # 206629 01 SD)
34	C	*Agrostis stolonifera L.*	Creeping Bentgrass	'Seaside' (Lot # 578530)
35	C		Creeping Bentgrass	'Southshore' (Lot # M2-8-511)
36	C		Creeping Bentgrass	'Penncross' (Lot # 3-2687)
37	C		Creeping Bentgrass	' Penn A-4' Lot # M2-8-531
38	C	*Agrostis stolonifera (palustris) L.*	Creeping Bentgrass	PI # 204390
39	C		Creeping Bentgrass	PI # 235541
55	C	*Agrostis castellana Bois. & Reut.*		Highland 2000
56	C			210428
57	C			240140
58	C			289644
59	C			Trust
60	C			469217B
64	C	*Agrostis vinealis*		PI # 440110
65	C			PI # 440110
66	C			PI # 440110
70	C	*Agrostis canina fascicularis*	Velvet Bentgrass	PI # 194697
71	C			PI # 194697
72	C			PI # 290707
80	C			SR
76	GT	*Agrostis trinii*		0-0-11-2
77	GT			0-0-11-2
78	GT			0-0-11-2
79	GT			0-0-11-2
73	C			PI # 598462
74	C			PI # 598462
75	C			PI # 598462
67	C	*Agrostis sp.*	Rhode Island Bentgrass	
68	C			
69	C			

Appendix VI

Response of bentgrass (*Agrostis* spp.) to postemergence herbicides

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Response of bentgrass (*Agrostis* spp.) to postemergence herbicides

Donald L. Suttner, Monsanto Company, St, Louis, MO

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Monsanto's glyphosate herbicides are used commercially to control bentgrass species in areas where they are undesirable such as on golf courses, lawn and landscapes, and turf seed and sod production fields. In addition, glyphosate is commonly used to kill existing bentgrass fairways and greens when renovation of these areas on golf courses is desired. The above uses exemplify two distinct patterns for use of herbicides to control a perennial species: 1) the control of occasional plants that may occur as undesirable species in other systems and 2) the control for established stands such as may be found in areas where the plant has been deliberately cultivated and must be subsequently removed prior to alternate use of the area. These use patterns may require different solutions due to the length and complexity of the stolon/rhizome mass in the latter system. Other methods of bentgrass control have also been employed. Mechanical removal of bentgrass patches by scalping with mowers and reseeding or by cutting out the sod and replacing with new sod are reported as effective options for bentgrass control in golf course roughs that adjoin creeping bentgrass fairways (Snow, 1982; Huber, 2002).

Currently, glyphosate resistant creeping bentgrass plants are being developed utilizing the *cp4 epsps* gene, which codes for an altered 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme (Lee, et al., 2002). This gene has also been utilized to develop glyphosate resistance in several agronomic crops including soybean, corn and cotton (Padgette et. al. 1996, Hart and Wax 1999). The development of Roundup tolerant turfgrasses was reported as a beneficial technology by Johnston et al. (1989). Roundup

tolerant cultivars of tall fescue (*Festuca arundinacea*) and hard fescue (*Festuca longifolia*) have been released for commercial use (Turf Seed, 2002).

The development and potential commercialization of glyphosate resistant creeping bentgrass poses a management challenge to identify alternative herbicides for control of glyphosate resistant bentgrass in areas where it is undesirable. The potential for unintended transfer of the glyphosate resistance gene to creeping bentgrass and related *Agrostis* species further encourages the need for control alternatives. Creeping bentgrass is a self-sterile, wind-pollinated, obligate out-crossing species and has the potential to transfer the glyphosate resistance trait via pollen to non-glyphosate resistant creeping bentgrass and related species (Davies 1953; Jones 1956a,b,c). Recent research with glufosinate resistant creeping bentgrass has shown that fertile hybrids with colonial bentgrass and dryland bentgrass that are glufosinate resistant can be obtained at very low frequencies in field conditions (Belanger et. al. 2003). More recently, studies by Christoffer (2003) and Wipff and Fricker (2001) have demonstrated that *Agrostis stolonifera* plants containing the *cp4 epsps* gene will similarly outcross to *A. stolonifera* and related species at low frequencies.

Herbicides in the cyclohexanedione and aryloxyphenoxy propionate families are commonly used to control both annual and perennial grasses in a wide variety of dicot agronomic crops (Ahrens 1994). Several of these herbicides such as fluazifop, clethodim and sethoxydim are also labeled for broadcast or directed applications in landscape planting beds and some turfgrass species for removal of unwanted annual and perennial grass species (BASF Corp. and MicroFlo Co. 2002; Valent USA Corp. 2002; Zeneca Ag Products 2001). Glyphosate controls plants by inhibiting the enzyme EPSP synthase involved in the synthesis of aromatic amino acids (Amrhein et al., 1980). Cyclohexanedione and arlyoxyphenoxy propionate herbicides control grass species by inhibiting the enzyme acetyl-CoA carboxylase (ACCase) that is involved in fatty acid biosynthesis (Burton et. al., 1989; Focke and Lichtenthaler, 1987). Another herbicide alternative that has potential for control glyphosate resistant creeping bentgrass and related species is glufosinate, which is a non-selective herbicide that controls plants by inhibiting the enzyme glutamine synthase resulting in a toxic accumulation of ammonia in plant cells (Logusch et al., 1991; Wild et al., 1987; Wild and Wendler, 1993).

There is little information currently available (Rely 24(c) label; UC Turf Sensitivity Guideline,1997; Nova Scotia, Canada, Environmental Horticulture Dept.) on the response of creeping bentgrass and related bentgrass species to applications of the cyclohexanedione herbicides such as clethodim and sethoxydim, the aryloxyphenoxy propionate herbicide such as fluazifop. Cook, *et al.* (1996) conducted a study that examined efficacy of glufosinate and glyphosate to control colonial bentgrass (*Agrostis capillaris* L.) turf in Oregon. Cook reported that 42 days after treatment, both products provided similar and complete control of the bentgrass. The objectives of this research were to evaluate the potential for these herbicides to control glyphosate tolerant and susceptible creeping bentgrass, as well as colonial, redtop, and dryland bentgrass in individual plant stands (Series I Experiments), to evaluate the parallel utility of these

products to control creeping bentgrass growing as a sod (Series II Experiments) and to further define the effects of plant age on susceptibility (Series III Experiments).

Series I Experiments

Experiments to evaluate control of individual bentgrass plants were conducted in 2001 at the Rutgers University Horticultural Research Farm II located in North Brunswick, New Jersey (NJ) and at the Scotts Company Research farm located in Marion County, Oregon (OR).

R1 Generation Plant Production of Transgenic Roundup Ready Creeping Bentgrass Events

R0 generation primary transformant plants from event ASR368, known to be hemizygous for the *cp4 epsps* gene, were field-transplanted in Marion County, OR during the fall of 1999. Floral induction and initiation occurred naturally under field conditions. Following induction, two vegetative clonal plants of event ASR368 were transferred into an isolated crossing block. Event ASR368 plants were surrounded by two plants each of five different non-transgenic Elite Parent Plant (EPP) creeping bentgrass selections. Plants were spaced on 3' centers.

Conventional lines represented in the crossing block with event ASR368 were designated as EPP vegetative lines V13-2-2, V15-2-5, V16-2-2, V10-1-8 and V14-2-6.

Fig. 1. Relative arrangement of year 2000 crossing block including event ASR 368 and V-series Elite Parent Plants.

V13-2-2	V10-1-8	V14-2-6	V15-2-5
V16-2-2	R0 ASR368	R0 ASR368	V16-2-2
V15-2-5	V14-2-6	V10-1-8	V13-2-2

The entire 3 plants x 4 plants crossing block of 12 plants was surrounded by waist high barriers consisting of hay bales and covered with Reemay® landscape fabric suspended by and secured to the hay bales during anthesis. Following anthesis, fabric was removed and plants were allowed to mature under natural conditions. Seedheads were hand harvested from each plant with knives when they reached maturity (50% of seedhead brown). Seedheads were placed in marked and labeled Kraft paper bags and dried in a greenhouse. Seed was then cleaned by hand on a screen dedicated to event ASR368, then purified with a General Blower (New Brunswick Sheet Metal Works, New Brunswick, NJ) set to an air flow opening of 8.5 for 2 minutes.

Seed harvested from only R0 plants of event ASR368 that were pollinated by the EPP plants were designated the R1 generation. R1 seed is expected to segregate 1:1 for event ASR368 Roundup Tolerant (RR) and event ASR368 Roundup Susceptible (RS)

phenotypes due to the hemizygous status of the *cp4 epsps* transgene in R0 plants and the strong self-incompatibility system that enforces cross pollination in creeping bentgrass. RR phenotypes in R1 seed are also expected to be hemizygous for the *cp4 epsps* transgene.

Events ASR365, ASR333 and ASR801 were each crossed in similar, but isolated crossing blocks and handled in a fashion similar to that of ASR368 in order to generate R1 seed.

R1 seed of each event was kept separate and germinated in a growth chamber. Seedlings were randomly transplanted to individual cells within Speedling® greenhouse flats . RR and RS seedlings were identified by the positive or negative results of a CP4 EPSPS protein immunoassay strip test conducted on each plant. Seedlings were transplanted to individual 3.5" plastic pots and labeled according to RR and RS during September, 2000. Plants were maintained in a contained facility in Marion County, OR during growth and development through May, 2001.

Reference Species Seed and Seedlings:

Seed of each non-transgenic reference species were germinated, transplanted and maintained using the same methods as for R1 seed of event ASR368. Commercial seed and plant introduction samples of the following *Agrostis* species were used to establish the non-transgenic reference species for the experiment:

Agrostis stolonifera: 'SR1020', 'Crenshaw', 'Penn A-4', 'Backspin'
Agrostis capillaris: 'SR7100', 'Bardot'
Agrostis castellana: 'Highland', 'Trust'
Agrostis gigantea: 'Streaker'

Plants were maintained in the contained facility until the shipping and transplanting dates.
Bentgrass plugs were transplanted into bare ground on May 9 and August 7, 2001 in New Jersey and on May 21 and August 16, 2001 in Oregon. The soil types were a Nixon loam with a pH of 6.3 and organic matter content of 3.0% in New Jersey and a Woodburn silt loam with a pH of 5.7 and organic matter content of 2.0% in Marion County, Oregon. Bentgrass species evaluated in studies at the first two locations included: event ASR368 Roundup Tolerant (RR); event ASR368 Roundup Susceptible (RS); a mixture of commercial creeping bentgrass hybrids ('Penn A-4', 'Backspin' and 'Crenshaw'); colonial bentgrass (*Agrostis capillaris* L. 'SR 7100'); redtop bentgrass (*Agrostis gigantea* With. 'Streaker'); and dryland bentgrass (*Agrostis castellana* Boiss. and Reut.'Trust').

Herbicide treatments consisted of glyphosate (Roundup PRO®[3]) at 1.6 kg ae/ha, glufosinate at 1.6 kg ai/ha, fluazifop at 0.3 and 0.4 kg ai/ha, clethodim at 0.3 kg ai/ha, sethoxydim at 0.5 kg ai/ha, and a combination of fluazifop and glyphosate applied 5 to 6 weeks after transplanting. Glufosinate was applied with spray grade ammonium sulfate at

[3] Roundup PRO is a registered trademark of Monsanto Technology, LLC

3.1 kg/ha. Fluazifop, clethodim, and sethoxydim treatments included crop oil concentrate (MSO-100, Ag Spray, Inc., Salem, OR) at 1.0% v/v. Herbicide treatments were applied with a single nozzle CO2 backpack sprayer equipped with a Teejet™[4] 9504E or 8004E spray tips (Spraying Systems Co., Wheaton, IL) delivering 40 GPA at 38 PSI.

Experimental sites were fertilized with 275 lbs/A of a 16-4-8 fertilizer blend at the New Jersey site and 275 lbs/A of a 16-16-16 fertilizer blend (Woodburn fertilizer, Woodburn, OR) at the Oregon site. Fertilization occurred at planting and again prior to herbicide application. Irrigation was applied as needed to supplement natural rainfall to help insure optimal growth of the bentgrass species.

Experimental design was a randomized complete block with three replications. Four bentgrass plugs of each species were planted equidistant in 6 feet long rows with rows spaced 2 feet apart. Each plant used during spring or late summer plantings had over 50 tillers and a well-developed root mass upon transplant. The commercial creeping bentgrass rows consisted of two plugs of 'Crenshaw' and one plug each of 'backspin' and 'Penn A-4'. Plants were allowed to acclimate to field conditions for at least 5 weeks under non-limiting nutrition, moisture and light conditions before the spray dates. Visual estimates of herbicide injury were taken at 2, 4 and 8 weeks after treatment (WAT) on a scale of 0 (no injury) to 100 (complete desiccation). Bentgrass diameters were measured immediately prior to herbicide application and at 8 WAT. The diameters of bentgrass plugs that were completely desiccated with no visual signs of regrowth were recorded as zero. Bentgrass diameter data at 8 weeks were converted to percent growth reduction by comparing the diameter of treated plants to the untreated checks. For purposes of clarity, experiments conducted with treatment dates in June and July are called 'summer season' and experiments conducted with treatment dates in September 'fall season'. All data was pooled across seasons and locations to test for significant season and location interactions. Bentgrass control and growth reduction data were analyzed as a two-factor (herbicide treatment by bentgrass species) factorial and means separated by the Fisher's Protected LSD test at the 0.05 probability level.

Results

Bentgrass Growth (Table 1). All untreated control plants increased in size from the time of treatment initiation to the termination of the experiment at 8 WAT. These data suggest that management and environmental conditions were conducive for bentgrass growth throughout the course of the experiment. However, the typical high temperatures and low humidity of the summer trial in Marion County, OR may have negatively affected results of all spray applications at this location.

Analysis of variance revealed significant season and location interactions for visual estimates of bentgrass control at 2 and 4 WAT. Therefore, data are presented separately for each experiment. Significant treatment by location interactions were also observed in the summer experiments for both visual estimates of bentgrass control and growth

[4] Teejet is a trademark of Spraying Systems Company

reduction data at 8 WAT. However, visual estimates of bentgrass control and growth reduction data were combined over locations for the fall experiments since no interaction was observed.

2 WAT (Table 2). Glyphosate treatment caused no visual injury to creeping bentgrass hybrid event ASR368 Roundup Tolerant (RR) in any experiment. Control of glyphosate susceptible bentgrass species with glyphosate or glufosinate was rapid, with control ranging from 88 to 99% with the exception of the summer experiment in OR where control of bentgrass with glyphosate ranged from 55 to 68%. No differential response was observed between glyphosate susceptible bentgrass species to glyphosate or glufosinate. Control of all bentgrass species, including RR, developed more slowly with fluazifop, clethodim, or sethoxydim compared to glyphosate and glufosinate. Control of susceptible bentgrass species with glyphosate + fluazifop was equal to glyphosate alone with the exception of the summer experiment in OR where control of colonial and red top bentgrass was lower with the combination of glyphosate plus fluazifop. No differences were observed between the response of RR and RS phenotypes in the response of any herbicide at 2 WAT.

4 WAT (Table 3). Control of glyphosate susceptible bentgrass species with glyphosate or glufosinate ranged from 88 to 99% with no differential response observed between glyphosate susceptible bentgrass species to either herbicide. Control of bentgrass species with fluazifop, clethodim, or sethoxydim ranged from 43 to 93% depending on experiment and herbicide treatment. In most cases, control of all glyphosate susceptible bentgrass species was lower with fluazifop, clethodim, or sethoxydim compared to glyphosate and glufosinate reflecting the slower onset of symptomology with the former chemistry. The lowest levels of bentgrass control with fluazifop, clethodim, and sethoxydim tended to be most prevalent on dryland and redtop bentgrass. Control of susceptible bentgrass species with glyphosate plus fluazifop was equal to glyphosate alone in all experiments. No differences were observed between the response of RR and RS phenotypes in the response of any herbicide at 4 WAT.

8 WAT (Table 4). Glyphosate caused no visual injury to RR phenotypes of event ASR368 in any experiment. Growth reduction data revealed that growth rates for RR treated with glyphosate were similar to untreated plants. Control of all glyphosate susceptible bentgrass lines with glyphosate was nearly complete in all experiments with no differential response between susceptible bentgrass lines observed.

Glufosinate provided 88 to 100% control of all bentgrass lines, including RR phenotype in the summer experiment in NJ and at both locations in the fall. Control of glyphosate susceptible bentgrass lines with glufosinate was equal to glyphosate in these three experiments except for dryland bentgrass in the summer experiment in NJ. However, all bentgrass lines treated with glufosinate in the summer experiment in OR showed significant regrowth as evidenced by both the visual estimates of bentgrass control and bentgrass growth reduction data. Control of redtop and dryland bentgrass with glufosinate was lower compared with creeping and colonial bentgrass in this experiment.

Fluazifop applied at 0.3 or 0.4 kg/ha provided growth reduction of RS phenotype and colonial bentgrass at levels equal to glyphosate in all experiments except for RR phenotype. Control of RR phenotype with fluazifop was similar to RS phenotype and colonial bentgrass. However, growth reduction of dryland bentgrass and redtop in the summer experiments was lower with fluazifop compared to glyphosate in some instances. There was no differential response between bentgrass species treated with 0.4 kg/ha of fluazifop in all experiments with the exception of the higher tolerance of dryland bentgrass observed in the summer experiment in NJ.

Clethodim provided control of glyphosate susceptible creeping and colonial bentgrass lines that was equal to glyphosate in the summer experiment in OR and the fall experiments at both locations. However, in the summer experiment in NJ control of RS phenotype with clethodim was lower compared to glyphosate. In some experiments, control of redtop and dryland bentgrass with clethodim was lower compared to glyphosate.

Sethoxydim provided 92 to 96% control of all bentgrass species in the fall experiments at both locations. For most bentgrass species control levels were equal to fluazifop and clethodim but less than glyphosate. In the summer experiments bentgrass control was lower with sethoxydim and in most cases, substantially lower than control levels obtained with glyphosate or fluazifop. In all experiments susceptible bentgrass control was equal for the glyphosate plus fluazifop treatment compared with glyphosate alone. In addition, control of RR phenotype with this combination was equal to fluazifop applied alone. No differences were observed between the response of RR phenotype and RS phenotype in the response of any herbicide at 8 WAT.

Conclusions

The results of these studies demonstrate that glufosinate and the ACCase inhibiting herbicides fluazifop, clethodim and sethoxydim have substantial herbicidal activity on creeping bentgrass and related species. No differential response between RR phenotype and RS phenotypes of creeping bentgrass to glufosinate or the ACCase inhibiting herbicides was observed in these experiments, confirming that, as in other crop species, the *cp4 epsps* gene conferring glyphosate resistance does not increase tolerance to herbicides that do not inhibit the EPSP synthase enzyme.

Control of glyphosate susceptible bentgrass with glyphosate was nearly complete in all experiments. Bentgrass species are generally considered to have a high level of tolerance to glyphosate relative to other perennial grass species as evidenced by the listing of only partial control on many glyphosate herbicide labels (Monsanto Co. 2002). The high levels of control of *Agrostis spp.* with glyphosate as well as the other herbicides may be indicative of the activity where treatments are applied to individual bentgrass plants leading to maximum herbicide contact with the bentgrass foliage and exposed stolons such as would be encountered with spot treatment in seed production or landscape beds. Further research was needed with these alternative herbicides to further evaluate their potential to control bentgrass on established bentgrass fairways and greens (mature sods).

Glufosinate provided rapid suppression of all bentgrass species and nearly complete control was maintained 8 WAT in 3 of 4 experiments. Substantial regrowth of all glufosinate treated bentgrass was observed in the summer experiment in OR and may be related to size of the bentgrass species prior to herbicide application or temperature/humidity conditions at this location. Bentgrass species prior to herbicide application were at least 50% larger and in some cases twice as large as the bentgrass species in the summer experiment in OR compared to the other three experiments (Table 1). In the summer experiment in OR average daily temperatures rapidly increased from the low 60's to the low 70's for several days prior to and immediately following herbicide application. Previous research with glufosinate has shown that control of weedy species may vary greatly depending on plant size and environmental conditions that may differ between locations, seasons and years (Steckel et al. 1997).

Herbicidal activity on bentgrass species with the ACCase inhibiting herbicides fluazifop, clethodim and sethoxydim was slower relative to glyphosate and glufosinate, but nearly complete control of all bentgrass species was observed at 8 WAT with fluazifop applied at 0.4 kg/ha. Control of all susceptible bentgrass species with this treatment was equal to glyphosate except for dryland bentgrass where control was slightly lower in some experiments. Bentgrass control with clethodim and sethoxydim was less consistent with substantial regrowth observed on all species except colonial bentgrass in the summer experiment in NJ for clethodim and in the summer experiments at both locations for sethoxydim. Previous research has shown that the activity of fluazifop and other ACCase inhibiting herbicides may be enhanced on grass species with the utilization of methylated seed oils in place of crop oil concentrates or with the addition of nitrogen sources (Nalewaja 1986, York et al. 1990). Further research should be conducted in this area to determine if bentgrass control can be further enhanced with ACCase herbicides.

Multiple applications of Roundup are typically required for complete control of creeping bentgrass (Crockett, 2002). Future research will also focus on sequential application of ACCase inhibiting herbicides to further enhance control of *Agrostis spp*.

Fluazifop appears to have the greatest potential to be utilized as an herbicide alternative for control of glyphosate tolerant bentgrass plants growing individually in areas where they are undesirable. Glufosinate and clethodim have also demonstrated substantial herbicidal activity on bentgrass species.

Series II Experiments

In view of the preceding results that RR and RS phenotypes of bentgrass were equally susceptible to alternative herbicide treatments, further research was conducted to assess the utility of the ACCase inhibitor herbicides in controlling mature sods of RS phenotypes of creeping bentgrass. Experiments were conducted at the Purdue University Turf Research Farm at West Lafayette, IN and the North Carolina State University Sandhills Turf Research Station at Jackson Springs, NC. Creeping bentgrass sod was established in fall, 2000 using the cultivar, Providence, in Indiana, whereas, sod was

established in 1995 using the cultivar, Penncross, in North Carolina. The soil types were a Starks-Fincastle silt loam with a pH of 7.2 in Indiana and a Candor sand with a pH of 6.1 in North Carolina.

Herbicide treatments consisted of glyphosate (Roundup PRO) at 1.7 kg ae/ha, fluazifop at 0.3 and 0.4 kg ae/ha, fluazifop at 0.4 kg ae/ha followed by 0.4 kg ae/ha as a sequential at 6 weeks after initial treatment, clethodim at 0.2 and 0.3 kg ae/ha, clethodim at 0.3 kg ae/ha followed by 0.3 kg ae/ha as a sequential at 6 weeks after initial treatment, sethoxydim at 0.4 kg ae/ha and sethoxydim at 0.4 kg ae/ha followed by 0.4 kg ae/ha as a sequential at 6 weeks after initial treatment. ACCase inhibitor herbicides were applied with Agridex™[5] COC (Helena Chemical Co., Memphis, TN) in North Carolina and with Krop Kare™[6] COC (Heartland Ag., Fort Dodge, IA) in Indiana at 1% volume/volume in both locations. Herbicide treatments were applied via compressed CO_2 powered sprayers in both locations. In Indiana, Teejet 8001.5 nozzles (Spraying Systems Co. Wheaton, IL) were used at 35 psi for a carrier volume of 814 l/ha. In North Carolina, Teejet 8002XR nozzles (Spraying Systems Co. Wheaton, IL) were used at 42 psi for a carrier volume of 304 l/ha.

Experimental design was a randomized complete block with three replications in Indiana and four replications in North Carolina. Plot size was 1.5 X 1.5 m in Indiana and 1.2 X 1.8 m in North Carolina.

Results

Creeping bentgrass control at 2 WAT (Table 5). Glyphosate provided 72 - 89% control of creeping bentgrass. As in the individual plant studies, onset of symptomology with ACCase herbicides was slower. Symptomology development with glyphosate was faster at the Indiana site than in North Carolina. Sequential applications had not been made at this application hence all ACCase herbicide treatments were providing similar control. All ACCase treatments were providing higher levels of control of creeping bentgrass in Indiana than in North Carolina at 2 WAT.

Creeping bentgrass control at 4 WAT (Table 6). Glyphosate provided excellent control of creeping bentgrass in North Carolina at 4 WAT. Onset of symptomology at this site continued to be slow with ACCase inhibitors. All treatments provided less activity than glyphosate at this rating and were similar to one another since no sequential applications had been made at 4WAT.

Creeping bentgrass control at 6 WAT (Table 7). Glyphosate provided a high level of control of creeping bentgrass at both locations at this rating. The ACCase inhibitors were not providing acceptable control of bentgrass at 6 WAT. Sequential applications of ACCase inhibitors were made after evaluation at this timing in both locations.

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[5] Agridex is a trademark of Helena Chemical Company
[6] Krop Kare is a trademark of Heartland Ag Inc.

Creeping bentgrass control at 8 WAT (Table 8). Glyphosate continued to provide a high level of control of creeping bentgrass in Indiana whereas in North Carolina, control had peaked at 6 WAT and was in decline. Excellent control levels were achieved with fluazifop, clethodim and sethoxydim when applied as sequentials at a six-week interval. Greater than 90 percent control was seen with all sequentials at both locations. No single application of an ACCase inhibitor to provide acceptable control at 8 WAT at either location. Both fluazifop and clethodim as sequentials were providing significantly higher levels of control than glyphosate in North Carolina at 8WAT.

Creeping bentgrass control at 12 WAT (Table 9). Sequential applications of the ACCase inhibitors continued to provide substantial levels of control of creeping bentgrass at both locations 12 WAT. Control with glyphosate had deteriorated significantly by this rating in North Carolina, however, it continued to perform well in the Indiana trial. Single applications of ACCase inhibitors were essentially providing no control at this rating.

Creeping bentgrass control at 16 WAT (Table 10). Fluazifop, clethodim and sethoxydim sequentials were providing more consistent control of creeping bentgrass at 4 months after initial treatment than was glyphosate when one considers performance across the two sites. By 16 WAT no treatment including glyphosate was providing consistent commercial control of bentgrass.

Conclusions

The results for studies on creeping bentgrass sod clearly demonstrate that it represents a more challenging weed control target than individual plants for both glyphosate and alternative control agents. Whereas results indicate that acceptable levels of control could be achieved with single applications of ACCase inhibitor herbicides on individual plants, sequential applications were needed to provide optimal results on sod. Similarly glyphosate provided good control with single plants but was inconsistent for controlling sod, consistent with label claims for this product. Results from these sod studies should be considered carefully since under normal practice tillage would be utilized in addition to herbicides to destroy creeping bentgrass sods in either a golf fairway/green, sod farm or seed production field. Tillage represents a substantial asset when combined with an effective herbicide program for eliminating any remaining vegetation not killed by the herbicide. The improved activity found with individual plants from a fall application suggests that the results from these studies with ACCase inhibitors on sod could be improved further through use of a fall-timed applications. The use of methylated seed oils and nitrogen sources as adjuvants to enhance performance will also be explored.

A combination treatment of glyphosate and fluazifop or clethodim appears to be compatible for use in site renovation to control mixed populations of glyphosate resistant and susceptible bentgrass, annual bluegrass, and broadleaf weed species. Research should continue with these herbicides to further evaluate bentgrass control with these herbicides applied as broadcast treatments to renovate golf course fairways and greens and as directed treatments to control bentgrass infestations in other desired turfgrasses.

Series III Experiments

The results of alternative control measures with individual plants and with sods suggest that several herbicides can be employed to control Roundup Ready creeping bentgrass. During 2002 additional studies were conducted to both confirm the results from previous years and further understand the effect of sod age on control level of RS phenotypes. Experiments were conducted during 2002 at the Purdue University Turf Research Farm (2 trials) at West Lafayette, IN, the North Carolina State University Sandhills Turf Research Station (2 trials) at Jackson Springs, NC, the University of Massachusetts Turf Research Station at Amherst, MA and the Washington State University Southwestern Agronomy Center at Puyallup, WA. Creeping bentgrass sod was established in 1995 in Indiana, in 1998 in North Carolina, the "early '90's" in Massachusetts and in the "mid '90's" in Washington.

Herbicide treatments consisted of glyphosate (Roundup PRO) at 1.7 kg ae/ha, fluazifop at 0.4 kg ai/ha, fluazifop at 0.4 kg ae/ha followed by 0.4 kg ae/ha as a sequential at 4 weeks after initial treatment, clethodim at 0.3 kg ae/ha, clethodim at 0.3 kg ae/ha followed by 0.3 kg ae/ha as a sequential at 4 weeks after initial treatment, sethoxydim at 0.4 kg ae/ha and sethoxydim at 0.4 kg ae/ha followed by 0.4 kg ae/ha as a sequential at 4 weeks after initial treatment. ACCase inhibitor herbicides were applied with crop oil concentrate at all locations at each application. Herbicide applications were applied via compressed gas powered sprayers at all locations. Spray tips and carrier volume were Teejet 8001 @ 407 l/ha in Indiana, Teejet VS8002 @ 32.5 gpa in North Carolina, Teejet VS11004 @ 50 gpa in Massachusetts and Teejet VS8003 @ 46 gpa in Washington.

Experimental design was a randomized complete block with 3 replications in Indiana, Massachusetts and Washington and 4 replications in North Carolina. Plot size was 5 X 5 ft in Indiana and Washington, 4 X 5 ft in North Carolina and 3.5 X 10 ft in Massachusetts.

Results

Creeping bentgrass control at 1 WAT (Table 11). Glyphosate provided 8 – 100% control of creeping bentgrass. Control of creeping bentgrass was substantially lower in North Carolina than in Massachusetts or Washington possibly due to the earlier time of applications and cooler temperatures. Consistent with 2001 work the ACCase inhibitor herbicides proved slower onset of symptomology at all sites. Only the effects of the first application were observable at this evaluation timing.

Creeping bentgrass control at 2 WAT (Table 12). Glyphosate provided 66 - 100% control of creeping bentgrass. Lowest control of creeping bentgrass was achieved in both trials North Carolina and was attributed to severe drought that affected much of North Carolina during 2003. Control with the ACCase inhibitors varied widely at this evaluation timing but generally ranged from the mid 40's to mid 90's in percent control. Sequential applications had not yet been applied and these materials show slower symptomology than glyphosate.

Creeping bentgrass control at 4 WAT (Table 13). Glyphosate provided near 100% control of creeping bentgrass except for one trial in North Carolina that was most effected by drought. All ACCase treatments provided less activity than glyphosate at this rating and were generally similar one to another in the absence of effects of a sequential applications

Creeping bentgrass control at 6 WAT (Table 14) Glyphosate provided 90 – 100% control of creeping bentgrass at most locations. At the North Carolina site that affected by severe drought, glyphosate activity had peaked and was declining at 6 WAT. Clethodim was providing excellent control with sequential applications even at the difficult North Carolina site. Sequential applications of Fluazifop were showing excellent activity except in the drought effected North Carolina trials. Sethoxydim sequential applications were also performing well except in North Carolina where it still trended better than Fluazifop.

Creeping bentgrass control at 8 WAT (Table 15) Glyphosate provided 61 – 99% control of creeping bentgrass and had peaked at most locations. Sequential applications of clethodim were providing 98 – 100% control in all trials and superior to glyphosate in the challenging conditions in North Carolina. Fluazifop and sethoxydim sequential applications were equivalent to glyphosate at most locations and trending better in North Carolina.

Creeping bentgrass control at 12 WAT (Table 16) Glyphosate provided 53 – 94% control of creeping bentgrass and was declining in all locations except Washington. Clethodim as a sequential application was showing continuing strength at this late rating and was equal to or better than glyphosate. Fluazifop sequential applications were equal to glyphosate at all locations. A trend toward less control with fluazifop in the Indiana trials was not detectable statistically due to the relatively large LSD's at this late evaluation. Control with sethoxydim was more variable and less than the other ACCase herbicides in Massachusetts and Washington.

Conclusions

The results of the 2002 studies confirm that on older sods and under drought stress the performance of a single application of glyphosate varies on creeping bentgrass. Under these stress conditions the ACCase inhibitor herbicides may also show less control. Clethodim generally provided the best control under these conditions and appears to be comparable to better than glyphosate under a drought scenario. The need for a sequential applications of ACCase herbicides to control a difficult species like creeping bentgrass is obvious from both 2001 and 2002 studies. Under drought stress a light tillage should be recommended after complete translocation of any of these products to provide complete control. Fluazifop and sethoxydim also show potential for creeping bentgrass control but in older sods would tend to consistently benefit from a light tillage flowing the second application to provide optimal results.

Overall, these studies demonstrate that there are several alternative herbicides that can control creeping bentgrass growing either as individual plants or in a sod. The use of these materials in tank mixes with glyphosate will provide broadspectrum weed control including Roundup Ready creeping bentgrass. The effect of these mixtures will in essence provide solutions that are equivalent or even better than those that were known before the advent of Roundup Ready creeping bentgrass. The ACCase inhibitor class of chemistry is large (Tomlin, 1997) and includes many commercial products that have not yet been investigated for activity on creeping bentgrass. These studies have focused on herbicides that are already labeled for key target sites and could be available for immediate use by the golf and grass seed production industries. Results from 2001 and 2002 show that age of sod and environmental conditions have no greater effect on the performance of alternative herbicides for the control of creeping bentgrass than they do on glyphosate.

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[7] PURE GOLD IS A TRADEMARK OF TURF-SEED, INC.

Table 1. Mean diameter size ± standard error of untreated bentgrass species prior to and 8 weeks following herbicide application.

Experiment	Bentgrass Species	Plant Diameter (cm)	
		0 WAT	8 WAT
New Jersey- Summer	RR 368[1]	20 (0.6)[2]	41 (2.7)
	RS 368	28 (0.5)	36 (2.7)
	CCB	23 (0.4)	41 (2.3)
	CLB	19 (0.3)	24 (1.1)
	RTB	17 (0.3)	32 (1.0)
	DLB	15 (0.5)	18 (0.7)
Oregon- Summer	RR 368	41 (1.6)	65 (3.4)
	RS 368	38 (1.4)	65 (3.4)
	CCB	47 (2.2)	67 (4.7)
	CLB	34 (0.8)	58 (0.6)
	RTB	34 (0.9)	56 (2.9)
	DLB	34 (1.1)	59 (2.6)
New Jersey- Fall	RR 368	12 (0.4)	17 (0.8)
	RS 368	12 (0.4)	16 (1.8)
	CCB	14 (0.5)	18 (2.6)
	CLB	13 (0.5)	20 (2.2)
	RTB	23 (0.5)	26 (0.9)
	DLB	15 (0.5)	23 (2.2)
Oregon- Fall	RR 368	26 (0.6)	49 (3.3)
	RS 368	25 (0.8)	47 (2.9)
	CCB	28 (0.9)	57 (6.5)
	CLB	21 (0.5)	26 (2.1)
	RTB	21 (1.1)	30 (3.7)
	DLB	24 (1.2)	43 (4.5)

[1] Bentgrass species: RR 368= Glyphosate resistant creeping bentgrass line 368; RS 368= Glyphosate susceptible creeping bentgrass line 368; CCB= Mixture of commercial creeping bentgrass 'Penn A-4', 'Backspin', and 'Crenshaw'; CLB= Colonial bentgrass 'SR-7100'; RTB= Red top bentgrass 'Streaker'; DLB= dryland bentgrass 'Trust'.

[2] Value in ( ) = standard error.

Table 2. Response of bentgrass species as individual plants to postemergence herbicides 2 WAT.

			----------% Control----------					
Experiment	Treatment	Rate kg/ha	RR[1] 368	RS 368	CCB	CLB	RTB	DLB
New Jersey- Summer	Glyphosate[2]	1.6	0	98	98	98	96	99
	Glufosinate[3]	1.6	99	99	98	99	96	99
	Fluazifop[4]	0.3	50	55	48	48	63	45
	Fluazifop	0.4	48	55	48	45	72	42
	Clethodim	0.3	50	55	52	53	60	42
	Sethoxydim	0.6	47	48	45	42	57	40
	Glyphosate + Fluazifop	1.6 + 0.4	0	96	98	95	98	98
	Check		0	0	0	0	0	0
	LSD $_{0.05}$		---------- 9 ----------					
Oregon- Summer	Glyphosate	1.6	0	55	60	65	68	67
	Glufosinate	1.6	99	99	99	99	99	99
	Fluazifop	0.3	27	20	27	10	15	13
	Fluazifop	0.4	30	30	25	13	12	17
	Clethodim	0.3	18	27	23	15	12	18
	Sethoxydim	0.6	23	23	32	15	13	10
	Glyphosate + Fluazifop	1.6 + 0.4	22	48	45	45	42	73
	Check		0	0	0	0	0	0
	LSD $_{0.05}$		---------- 15 ----------					
New Jersey- Fall	Glyphosate	1.6	0	93	95	96	95	96
	Glufosinate	1.6	94	94	94	97	93	97
	Fluazifop	0.3	47	50	53	50	48	45
	Fluazifop	0.4	48	50	55	58	50	47
	Clethodim	0.3	45	48	43	57	45	47
	Sethoxydim	0.6	45	48	50	47	47	45
	Glyphosate + Fluazifop	1.6 + 0.4	53	91	96	96	95	95
	Check		0	0	0	0	0	0
	LSD $_{0.05}$		---------- 7 ----------					
Oregon- Fall	Glyphosate	1.6	0	96	90	92	88	92
	Glufosinate	1.6	97	98	98	96	97	98
	Fluazifop	0.3	43	40	32	40	42	42
	Fluazifop	0.4	38	38	42	27	42	40
	Clethodim	0.3	42	45	40	40	42	42
	Sethoxydim	0.6	35	40	37	37	47	40
	Glyphosate + Fluazifop	1.6 + 0.4	35	92	83	92	92	94
	Check		0	0	0	0	0	0
	LSD $_{0.05}$		---------- 9 ----------					

[1]Bentgrass species: RR 368= Glyphosate resistant creeping bentgrass line 368; RS 368= Glyphosate susceptible creeping bentgrass line 368; CCB= Mixture of commercial creeping bentgrass 'Penn A-4', 'Backspin', and 'Crenshaw'; CLB= Colonial bentgrass 'SR-7100'; RTB= Red top bentgrass 'Streaker'; DLB= dryland bentgrass 'Trust'.
[2]Glyphosate applied as Roundup Pro formulation.
[3]Glufosinate applied with spray grade ammonium sulfate at 3.1 kg/ha.
[4]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.

Table 3. Response of bentgrass species as individual plants to postemergence herbicides 4 WAT.

			----------% Control----------					
Experiment	Treatment	Rate kg/ha	RR[1] 368	RS 368	CCB	CLB	RTB	DLB
New Jersey- Summer	Glyphosate	1.6	0	99	99	99	99	99
	Glufosinate	1.6	99	99	98	99	98	96
	Fluazifop	0.3	85	85	87	85	89	87
	Fluazifop	0.4	87	87	87	82	92	90
	Clethodim	0.3	78	77	80	85	87	83
	Sethoxydim	0.6	78	75	72	83	87	73
	Glyphosate + Fluazifop	1.6 + 0.4	85	98	99	99	99	99
	Check		0	0	0	0	0	0
	LSD $_{0.05}$		---------- 6 ----------					
Oregon- Summer	Glyphosate[2]	1.6	0	98	98	98	98	98
	Glufosinate[3]	1.6	93	91	96	93	88	94
	Fluazifop[4]	0.3	82	90	93	62	55	38
	Fluazifop	0.4	88	85	91	82	77	52
	Clethodim	0.3	80	83	85	63	68	47
	Sethoxydim	0.6	75	75	73	75	50	43
	Glyphosate + Fluazifop	1.6 + 0.4	80	97	98	98	97	98
	Check		0	0	0	0	0	0
	LSD $_{0.05}$		---------- 13 ----------					
New Jersey- Fall	Glyphosate	1.6	0	99	99	99	99	99
	Glufosinate	1.6	96	97	99	99	97	99
	Fluazifop	0.3	85	88	91	91	83	85
	Fluazifop	0.4	90	91	93	94	80	85
	Clethodim	0.3	87	89	80	91	73	75
	Sethoxydim	0.6	86	87	83	86	75	75
	Glyphosate + Fluazifop	1.6 + 0.4	92	98	99	99	99	99
	Check		0	0	0	0	0	0
	LSD $_{0.05}$		---------- 8 ----------					
Oregon- Fall	Glyphosate	1.6	0	98	98	98	98	98
	Glufosinate	1.6	97	98	98	97	98	98
	Fluazifop	0.3	70	73	60	67	65	72
	Fluazifop	0.4	70	63	65	57	62	65
	Clethodim	0.3	77	78	75	85	63	73
	Sethoxydim	0.6	68	77	72	67	58	60
	Glyphosate + Fluazifop	1.6 + 0.4	78	98	98	98	98	98
	Check		0	0	0	0	0	0
	LSD $_{0.05}$		---------- 12 ----------					

[1]Bentgrass species: RR 368= Glyphosate resistant creeping bentgrass line 368; RS 368= Glyphosate susceptible creeping bentgrass line 368; CCB= Mixture of commercial creeping bentgrass 'Penn A-4', 'Backspin', and 'Crenshaw'; CLB= Colonial bentgrass 'SR-7100'; RTB= Red top bentgrass 'Streaker'; DLB= dryland bentgrass 'Trust'.
[2]Glyphosate applied as Roundup Pro formulation.
[3]Glufosinate applied with spray grade ammonium sulfate at 3.1 kg/ha.
[4]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.

Table 4. Response of bentgrass species as individual plants to postemergence herbicides 8WAT.

			% Control						% Growth Reduction[1]					
Experiment	Treatment	Rate kg/ha	RR[2] 368	RS 368	CCB	CLB	RTB	DLB	RR 368	RS 368	CCB	CLB	RTB	DLB
New Jersey- Summer	Glyphosate[3]	1.6	0	100	100	100	100	100	8	100	100	100	100	100
	Glufosinate[4]	1.6	93	93	93	100	96	88	97	97	95	99	98	82
	Fluazifop[5]	0.3	88	93	95	96	90	88	89	93	97	97	91	72
	Fluazifop	0.4	95	95	95	98	98	91	98	96	95	99	97	80
	Clethodim	0.3	72	73	77	93	73	77	80	77	83	96	81	88
	Sethoxydim	0.6	70	68	63	88	82	58	81	69	75	85	83	34
	Glyphosate + Fluazifop	1.6 + 0.4	96	100	100	100	100	100	97	100	100	100	100	100
	Check		0	0	0	0	0	0	0	0	0	0	0	0
	$LSD_{0.05}$		9						9					
Oregon- Summer	Glyphosate	1.6	0	100	99	100	98	98	0	100	99	100	99	99
	Glufosinate	1.6	60	55	65	70	40	53	43	36	64	73	29	57
	Fluazifop	0.3	93	97	98	98	98	88	88	90	98	99	99	95
	Fluazifop	0.4	95	96	100	100	99	93	98	97	100	100	93	99
	Clethodim	0.3	95	92	96	98	95	83	94	91	96	99	93	88
	Sethoxydim	0.6	72	73	83	92	77	43	74	70	81	93	76	44
	Glyphosate + Fluazifop	1.6 + 0.4	96	100	100	99	100	100	99	100	100	99	100	100
	Check		0	0	0	0	0	0	0	0	0	0	0	0
	$LSD_{0.05}$		8						12					
New Jersey /Oregon- Fall	Glyphosate	1.6	0	100	100	100	100	100	4	100	100	100	100	100
	Glufosinate	1.6	95	98	98	99	98	100	93	93	97	99	98	100
	Fluazifop	0.3	97	97	94	98	97	98	98	98	98	99	99	99
	Fluazifop	0.4	96	96	97	97	96	95	99	98	99	99	99	99
	Clethodim	0.3	97	96	96	98	93	96	96	93	98	99	96	97
	Sethoxydim	0.6	95	95	94	96	92	93	98	96	98	99	96	97
	Glyphosate + Fluazifop	1.6 + 0.4	97	100	100	100	100	100	99	100	100	100	100	100
	Check		0	0	0	0	0	0	0	0	0	0	0	0
	$LSD_{0.05}$		5						8					

[1] Percent growth reduction calculated by comparing the diameter of treated bentgrass plants to untreated controls.
[2] Bentgrass species: RR 368= Glyphosate resistant creeping bentgrass line 368; RS 368= Glyphosate susceptible creeping bentgrass line 368; CCB= Mixture of commercial creeping bentgrass 'Penn A-4', 'Backspin', and 'Crenshaw'; CLB= Colonial bentgrass 'SR-7100'; RTB= Red top bentgrass 'Streaker'; DLB= dryland bentgrass 'Trust'.
[3] Glyphosate applied as Roundup Pro formulation.
[4] Glufosinate applied with spray grade ammonium sulfate at 3.1 kg/h.
[5] Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.

Table 5. Response of creeping bentgrass as a sod to postemergence herbicides 2 WAT.

		----------% Control --------	
Treatment	Rate kg/ha	Indiana	North Carolina
Glyphosate[1]	1.6	89	74
Fluazifop[2]	0.3	70	29
Fluazifop	0.4	67	35
Fluazifop + Fluazifop[3]	0.4 + 0.4	63	33
Clethodim[2]	0.2	56	34
Clethodim	0.3	67	31
Clethodim + Clethodim[3]	0.3 + 0.3	67	35
Sethoxydim[2]	0.4	89	29
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	89	29
Checks		0	0
LSD (.05)		11.1	10.8

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 6 weeks after initial application.

Table 6. Response of creeping bentgrass as a sod to postemergence herbicides 4 WAT.

		----------% Control----------	
Treatment	Rate kg/ha	Indiana[4]	North Carolina
Glyphosate[1]	1.6		94
Fluazifop[2]	0.3		30
Fluazifop	0.4		55
Fluazifop + Fluazifop[3]	0.4 + 0.4		46
Clethodim[2]	0.2		26
Clethodim	0.3		38
Clethodim + Clethodim[3]	0.3 + 0.3		44
Sethoxydim[2]	0.4		31
Sethoxydim + Sethoxydim[3]	0.4 + 0.4		31
Checks			0
LSD (.05)			24.2

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 6 weeks after initial application.
[4]No 4 WAT evaluation in Indiana.

Table 7. Response of creeping bentgrass as a sod to postemergence herbicides 6 WAT.

		----------% Control----------	
Treatment	Rate kg/ha	Indiana	North Carolina
Glyphosate[1]	1.6	100	90
Fluazifop[2]	0.3	13	16
Fluazifop	0.4	38	54
Fluazifop + Fluazifop[3]	0.4 + 0.4	31	36
Clethodim[2]	0.2	9	21
Clethodim	0.3	11	25
Clethodim + Clethodim[3]	0.3 + 0.3	13	26
Sethoxydim[2]	0.4	91	26
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	100	18
Checks		0	0
LSD (.05)		23.6	23.9

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 6 weeks after initial application.

Table 8. Response of creeping bentgrass as a sod to postemergence herbicides 8 WAT.

		----------% Control----------	
Treatment	Rate kg/ha	Indiana	North Carolina
Glyphosate[1]	1.6	99	81
Fluazifop[2]	0.3	9	10
Fluazifop	0.4	20	24
Fluazifop + Fluazifop[3]	0.4 + 0.4	99	94
Clethodim[2]	0.2	4	13
Clethodim	0.3	5	15
Clethodim + Clethodim[3]	0.3 + 0.3	99	100
Sethoxydim[2]	0.4	99	10
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	100	93
Check		0	0
LSD (.05)		20.9	12.6

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 6 weeks after initial application.

Table 9. Response of creeping bentgrass as a sod to postemergence herbicides 12 WAT.

		----------% Control----------	
Treatment	Rate kg/ha	Indiana	North Carolina
Glyphosate[1]	1.6	98	56
Fluazifop[2]	0.3	2	1
Fluazifop	0.4	0	4
Fluazifop + Fluazifop[3]	0.4 + 0.4	86	84
Clethodim[2]	0.2	0	0
Clethodim	0.3	0	4
Clethodim + Clethodim[3]	0.3 + 0.3	93	89
Sethoxydim[2]	0.4	96	3
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	100	81
Checks		0	0
LSD (.05)		4.7	14.9

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 6 weeks after initial application.

Table 10. Response of creeping bentgrass as a sod to postemergence herbicides 16 WAT.

		----------% Control----------	
Treatment	Rate kg/ha	Indiana	North Carolina
Glyphosate[1]	1.6	97	41
Fluazifop[2]	0.3	0	0
Fluazifop	0.4	0	1
Fluazifop + Fluazifop[3]	0.4 + 0.4	83	75
Clethodim[2]	0.2	0	0
Clethodim	0.3	0	1
Clethodim + Clethodim[3]	0.3 + 0.3	83	78
Sethoxydim[2]	0.4	95	1
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	98	66
Check		0	0
LSD (.05)		9.6	18.8

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 6 weeks after initial application.

Table 11. Response of creeping bentgrass as a 5+-year-old sod to postemergence herbicides 1 WAT[4].

		----------	----------% Control-------	----------			
Treatment	Rate kg/ha	Indiana	Indiana	North Carolina	North Carolina	Massachusetts	Washington
Glyphosate[1]	1.6	-	-	8	-	100	100
Fluazifop[2]	0.4	-	-	5	-	-	37
Fluazifop + Fluazifop[3]	0.4 + 0.4	-	-	5	-	75	40
Clethodim	0.3	-	-	4	-	-	37
Clethodim + Clethodim[3]	0.3 + 0.3	-	-	5	-	82	37
Sethoxydim[2]	0.4	-	-	5	-	-	30
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	-	-	5	-	82	33
Check		-	-	0	-	0	0
LSD (.05)		-	-	3.4	-	18.2	5

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 4 weeks after initial application.
[4]WAT is weeks after initial treatment.

Table 12. Response of creeping bentgrass as a 5+-year-old sod to postemergence herbicides 2 WAT[4].

		----------	----------	----------% Control-------	----------	----------	----------
Treatment	Rate kg/ha	Indiana	Indiana	North Carolina	North Carolina	Massachusetts	Washington
Glyphosate[1]	1.6	100	100	66	74	100	100
Fluazifop[2]	0.4	66	-	26	44	-	95
Fluazifop + Fluazifop[3]	0.4 + 0.4	70	30	19	39	95	93
Clethodim	0.3	35	-	16	49	-	95
Clethodim + Clethodim[3]	0.3 + 0.3	37	37	21	50	93	95
Sethoxydim[2]	0.4	35	-	20	38	-	95
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	37	42	11	53	94	95
Check		0	0	0	0	0	0
LSD (.05)		19.4	13.4	18.9	30.2	8.3	2

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 4 weeks after initial application.
[4]WAT is weeks after initial treatment.

Table 13. Response of creeping bentgrass as a 4+-year-old sod to postemergence herbicides 4 WAT[4].

		----------	----------% Control--------	----------			
Treatment	Rate kg/ha	Indiana	Indiana	North Carolina	North Carolina	Massachusetts	Washington
Glyphosate[1]	1.6	100	99	97	63	100	100
Fluazifop[2]	0.4	43	-	54	35	-	98
Fluazifop + Fluazifop[3]	0.4 + 0.4	75	75	46	36	99	99
Clethodim	0.3	18	-	25	35	-	100
Clethodim + Clethodim[3]	0.3 + 0.3	27	27	31	34	97	100
Sethoxydim[2]	0.4	33	-	38	33	-	99
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	43	43	23	33	82	100
Check		0	0	0	0	0	0
LSD (.05)		25.6	19.7	22.1	24.3	21.4	2

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 4 weeks after initial application.
[4]WAT is weeks after initial treatment.

Table 14. Response of creeping bentgrass as a 4+-year-old sod to postemergence herbicides 6 WAT[4].

		----------	----------	----------% Control-------	----------	----------	----------
Treatment	Rate kg/ha	Indiana	Indiana	North Carolina	North Carolina	Massachusetts	Washington
Glyphosate[1]	1.6	100	100	90	56	100	98
Fluazifop[2]	0.4	47	-	40	44	-	100
Fluazifop + Fluazifop[3]	0.4 + 0.4	100	100	71	80	99	100
Clethodim	0.3	12	-	14	28	-	99
Clethodim + Clethodim[3]	0.3 + 0.3	100	100	86	94	100	98
Sethoxydim[2]	0.4	42	-	16	21	-	90
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	98	98	73	88	97	90
Check		0	0	0	0	0	0
LSD (.05)		34.5	37.4	22.9	25.5	14.0	7

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 4 weeks after initial application.
[4]WAT is weeks after initial treatment.

Table 15. Response of creeping bentgrass as a 4+-year-old sod to postemergence herbicides 8 WAT[4].

		----------	----------% Control-------	----------			
Treatment	Rate kg/ha	Indiana	Indiana	North Carolina	North Carolina	Massachusetts	Washington
Glyphosate[1]	1.6	97	98	75	61	99	92
Fluazifop[2]	0.4	22	-	49	51	-	70
Fluazifop + Fluazifop[3]	0.4 + 0.4	96	96	71	92	99	87
Clethodim	0.3	15	-	11	43	-	100
Clethodim + Clethodim[3]	0.3 + 0.3	99	99	98	98	100	100
Sethoxydim[2]	0.4	40	-	21	50	-	100
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	94	94	83	98	98	87
Check		0	0	0	0	0	0
LSD (.05)		28.9	38.9	25.0	25.0	16.9	27

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 4 weeks after initial application.
[4]WAT is weeks after initial treatment.

Table 16. Response of creeping bentgrass as a 4+-year-old sod to postemergence herbicides 12 WAT[4].

		----------	----------	----------% Control-------	----------	----------	----------
Treatment	Rate kg/ha	Indiana	Indiana	North Carolina	North Carolina	Massachusetts	Washington
Glyphosate[1]	1.6	85	94	53	-	87	92
Fluazifop[2]	0.4	17	-	48	-	-	85
Fluazifop + Fluazifop[3]	0.4 + 0.4	70	70	55	-	88	96
Clethodim	0.3	9	-	8	-	-	47
Clethodim + Clethodim[3]	0.3 + 0.3	78	78	79	-	99	100
Sethoxydim[2]	0.4	20	-	15	-	-	47
Sethoxydim + Sethoxydim[3]	0.4 + 0.4	69	69	76	-	58	86
Check		0	0	0	-	0	0
LSD (.05)		37.1	41.4	27.0	-	21.7	17.0

[1]Glyphosate applied as Roundup Pro formulation.
[2]Fluazifop, clethodim, and sethoxydim applied with crop oil concentrate at 1.0% v/v.
[3]Sequential application 4 weeks after initial application.
[4]WAT is weeks after initial treatment.

Appendix VII

Response to postemergence herbicides of hybrids derived from Roundup Ready creeping bentgrass (*Agrostis spp.*) interspecific/intergeneric outcrosses

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Response to postemergence herbicides of hybrids derived from Roundup Ready creeping bentgrass (*Agrostis spp.*) interspecific/intergeneric outcrosses

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Creeping bentgrass (*Agrostis stolonifera*) is a self-sterile, wind pollinated, obligate outcrossing species (Davies 1953; Jones 1956a,b,c). Interspecific/intergeneric outcrossing with creeping bentgrass has the potential to occur. Recent research with glufosinate resistant creeping bentgrass has shown that fertile hybrids with colonial bentgrass and dryland bentgrass that are glufosinate resistant can be obtained at very low frequencies in field conditions (Belanger et. al. 2003). More recently, studies by Christoffer (2003) have demonstrated that *Agrostis stolonifera* plants containing the *cp4 epsps* gene will similarly outcross to *A. stolonifera* and related species at low frequencies. Such hybrids are likely to exist in nature presently since interspecific/intergeneric outcrossing is not unique to transgenic creeping bentgrass. Hybrids derived from crosses with Roundup Ready creeping bentgrass can contain the *cp4 epsps* gene. It is desirable to determine if postemergence herbicides that have been shown to be effective in controlling Roundup Ready creeping bentgrass can also be employed to control hybrids containing the transgene. Other options such as mechanical removal are obvious alternatives to use of herbicides to remove these plants where they may be undesirable. To the extent that these plants might occur in wild areas where herbicide use may be deemed undesirable, the latter methods may be particularly well suited. It is observed, however, that under this scenario it is unlikely that such hybrids will be conspicuous as containing the Roundup Ready gene since they are, in all other respects, identical to non-transgenic hybrids.

Herbicides in the cyclohexanedione and aryloxyphenoxy propionate families have been show to control individual plants and sods of creeping bentgrass (Suttner et al. 2003). Cyclohexanedione and arlyoxyphenoxy propionate herbicides control grass species by inhibiting the enzyme acetyl-CoA carboxylase (ACCase), which is involved in fatty acid biosynthesis (Burton et. al., 1989; Focke and Lichtenthaler, 1987). Another herbicide alternative that has potential for control glyphosate resistant creeping bentgrass and related species is glufosinate, which is a non-selective herbicide that controls plants by inhibiting the enzyme glutamine synthase resulting in a toxic accumulation of ammonia in plant cells (Logusch et al., 1991; Wild et al., 1987; Wild and Wendler, 1993).

Since no information currently exists on the response of these interspecific/intergeneric hybrids to herbicides in the above classes, research was conducted during 2002 to understand the utility of these products for control of hybrids.

Spring 2002 Experiment

An experiment to evaluate control of hybrids grown as individual plants was conducted in the poly-house at Marion County, OR, during spring, 2002. Hybrids were derived either from forced crosses in the greenhouse or from seed obtained in field experiments (Christoffer 2003). The trial was established on March 1, 2002, by planting seedlings germinated in peat pellets in pre-formed holes in flats containing soil. Fertilizer was applied prior to seeding as 16-16-16 at a rate of 1.0 lbs of N/1000 sq ft. using an SR 2000 spreader with cone 8 at setting R ½. The trial was installed using a randomized complete block design with 3 replications.

Hybrids included in this trial were crosses of Roundup Ready creeping bentgrass (*Agrostis stolonifera*) with *A gigantea, A. idahoensis, A. pallens, A. capillaris, Polypogan monspeliensis, P. fugax and P. viridis*. One to four hybrid accessions were examined. These accessions were compared within the experiment for control with their respective parents where possible (ASR 368 and the above compatible species). Also included for comparison were the susceptible commercial creeping bentgrass cultivars Southshore, Crenshaw, Backspin, Penneagle and Penn A-4.

Spray applications were made on May 1, 2002, to two-month-old plants. Herbicide treatments consisted of glyphosate (Roundup PRO®[8]) at 1.6 kg ae/ha, glufosinate at 1.6 kg ai/ha, fluazifop at 0.4 kg ai/ha, sethoxydim at 0.5 kg ai/ha. Glufosinate was applied with spray grade ammonium sulfate at 3.1 kg/ha. Fluazifop and sethoxydim treatments included crop oil concentrate (MSO-100, Ag Spray, Inc., Salem, OR) at 1.0% v/v. Herbicide treatments were applied with a single nozzle CO2 backpack sprayer equipped with a Teejet™[9] 8004E spray tips (Spraying Systems Co., Wheaton, IL**)** delivering 40 GPA at 38 PSI.

Plants designated as "RR" are hybrids of the respective species with the exception of ASR 368. Plants designated as "RS" are susceptible parent species or non-transgenic cultivars. Evaluations for control were made at 7, 14, 28 and 42 days after application (DAA) using a 0 to 5 scale where 5 equals compete control and 0 equals no control. An evaluation of percent regrowth was also made at the 42 DAA rating date.

Results

Glyphosate provided complete control of susceptible *A. gigantea* at 28-42 DAA (Table 1). It provided no activity on either of the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop provided commercial control of both hybrids and parent by 42 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim also provided commercial control of *A. gigantea* hybrids by 42 DAA although control tended to be slightly lower than with Fluazifop. This mirrors the results from creeping bentgrass mitigation studies. Glufosinate provided near-complete burndown of both

[8] Roundup PRO is a registered trademark of Monsanto Technology, LLC
[9] Teejet is a trademark of Spraying Systems Company

hybrids and parent by 28 DAA however control had peaked at this rating and control was in decline by 42 DAA.

Glyphosate provided no activity on either of the hybrid accessions of *A. idahoensis* indicating presence of the *cp4 epsps* gene (Table 2). Parent germplasm of this species was unavailable for this experiment. Fluazifop provided commercial control of both hybrids and parent by 42 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim also provided commercial control of *A. idahoensis* hybrids by 42 DAA although control tended to be slightly lower than with Fluazifop similar to the results from creeping bentgrass mitigation studies. Glufosinate provided near-complete burndown of both hybrids by 28 DAA and control was maintained at a high level through 42 DAA. No regrowth of *A. idahoensis* was observed with this treatment.

Glyphosate provided no activity on either of the hybrid accession of *A. pallens* indicating presence of the *cp4 epsps* gene (Table 3). Parent germplasm of this species was unavailable for this experiment. Fluazifop provided commercial control of the hybrid by 42 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim also provided commercial control of *A. pallens* hybrids by 42 DAA although control tended to be slightly lower than with Fluazifop. Glufosinate provided near-complete burndown of both hybrids and parent by 28 DAA however control had peaked at this rating and control was declining by 42 DAA.

Glyphosate provided complete control of susceptible *P. monspeliensis* at 14-42 DAA (Table 4). It provided little activity on either of the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop provided a high level of control of both hybrids and parent by 42 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim was equally effective in control of *P. monspeliensis* hybrids by 42 DAA. Glufosinate provided near-complete burndown of both hybrids by 28 DAA however control had peaked at this rating and control was in decline by 42 DAA. The high level of control with glufosinate observed at 28 DAA persisted through 42 DAA evaluations with the parent plants.

Glyphosate provided complete control of susceptible *P. fugax* at 14-42 DAA (Table 5). It provided little activity on the hybrid accession indicating presence of the *cp4 epsps* gene. Fluazifop provided a high level of control of the hybrid and parent by 42 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim was equally effective in control of the *P. fugax* hybrid by 42 DAA. Glufosinate provided near-complete burndown of the hybrid and parent by 28 DAA, however control had peaked at this rating and control was in decline by 42 DAA.

Glyphosate provided complete control of susceptible *P. viridis* at 28-42 DAA (Table 6). It provided no activity on the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop provided commercial control of the hybrid and parent by 42 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim was equally effective in control of the *P. viridis* hybrid by 42 DAA. Glufosinate provided near-complete burndown of the hybrid by 28 DAA, however control had peaked at this

rating and was in decline by 42 DAA. The high level of control with glufosinate observed at 28 DAA persisted through 42 DAA evaluations with the parent plants.

Glyphosate provided complete control of susceptible *A. capillaris* at 28-42 DAA (Table 7). It provided little activity on either of the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop provided a high level of control of all three hybrids and parent by 42 DAA. The effects were slower to develop than with glyphosate. Sethoxydim was less effective in control of two of three *A. capillaris* hybrids by 42 DAA. The reduced effectiveness of sethoxydim was also apparent with the susceptible parents. Glufosinate provided near-complete burndown of all hybrids by 28 DAA however control had peaked at this rating and control was in decline by 42 DAA. The high level of control with glufosinate observed at 28 DAA persisted through 42 DAA evaluations with the parent plants.

Glyphosate provided complete control of susceptible commercial cultivars at 28-42 DAA (Table 8). Fluazifop provided a high level of control of all commercial cultivars by 42 DAA. The effects were slower to develop than with glyphosate. Sethoxydim was equally effective in control of two of the commercial cultivars by 42 DAA. Glufosinate provided near-complete burndown of commercial cultivars by 28 DAA however control had peaked at this rating and control was in decline by 42 DAA.

Glyphosate provided no activity on ASR 368 due to the presence of the *cp4 epsps* gene (Table 9). Fluazifop provided a high level of control of ASR 368 by 42 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim also provided equivalent control of ASR 368 by 42 DAA. Glufosinate provided near-complete burndown of ASR 368 by 28 DAA and control was maintained at a high level through 42 DAA.

Conclusions

The spring, 2002, experiment demonstrated that the ACCase inhibitor herbicides can provide similar solutions for the control of hybrids formed by *A. stolonifera* in interspecific/intergeneric crosses as they do for control of volunteers of Roundup Ready creeping bentgrass. These hybrids when treated in the spring respond as individual plants of creeping bentgrass and do not require a sequential application of herbicide for removal from plant communities. A spot treatment is likely the preferred method for removal of an occasional plant. Glufosinate, while showing some promise for control of hybrids, will require repeat applications to provide commercial levels of control. No differences were observed at this timing between hybrids containing *cp4 epsps* and their susceptible parent.

<u>Fall 2002 Experiment</u>

A second experiment to evaluate control of hybrids grown as individual plants was conducted in the greenhouse at the Marion County, Oregon poly-house during fall, 2002. Hybrids were again derived either from forced crosses in the greenhouse or from seed

obtained in field experiments (Christoffer 2003). The trial was established on August 15/16, 2002, by planting seedlings germinated in peat pellets in pre-formed holes in flats containing soil. Fertilizer was applied prior to seeding as 16-16-16 at a rate of 1.0 lbs of N/1000 sq ft. using an SR 2000 spreader with cone 8 at setting R ½. The trial was installed using a randomized complete block design with 3 replications.

Hybrids included in this trial were crosses of Roundup Ready creeping bentgrass (*Agrostis stolonifera*) with *A gigantea, A. idahoensis, A. pallens, A. capillaries A. pallida A. trinii, Polypogan monspeliensis, P. fugax and P. viridis.* One or more accessions were examined from each cross. These accessions were compared within the experiment for control with their respective parents (the above compatible species) where possible. Also included for comparison were the susceptible commercial creeping bentgrass cultivars "Seaside", "Southshore", "Penncross", and "L-93". Included, as an additional comparison was *A. stolonifera var. "Palustris"*. Further comparisons included susceptible *A. castellana and A. canina*.

Spray applications were made on October 22, 2002, to nine-week-old plants. Herbicide treatments consisted of glyphosate (Roundup PRO®[10]) at 1.6 kg ae/ha, glufosinate at 1.6 kg ai/ha, fluazifop at 0.4 kg ai/ha, sethoxydim at 0.5 kg ai/ha. Glufosinate was applied with spray grade ammonium sulfate at 3.1 kg/ha. Fluazifop and sethoxydim treatments included crop oil concentrate (MSO-100, Ag Spray, Inc., Salem, OR) at 1.0% v/v. Herbicide treatments were applied with a single nozzle CO2 backpack sprayer equipped with a Teejet™[11] 8004E spray tips (Spraying Systems Co., Wheaton, IL**)** delivering 40 GPA at 38 PSI.

Plants designated as "RR" are hybrids of the respective species with the exception of ASR 368. Plants designated as "RS" are susceptible parent species or non-transgenic cultivars. Evaluations for control were made at 16, 31and 45 days after application (DAA) using a 0-5 scale where 5 equals compete control and 0 equals no control.

Results

Glyphosate provided only marginal and commercially unacceptable control of susceptible *A. gigantea* at 45 DAA in the fall experiment (Table 10). It provided little activity on any of the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop also provided marginal and commercially unacceptable control of both hybrids and parent by 45 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim provided similar activity as fluazifop on *A. gigantea* hybrids and parents by 45 DAA although control tended to be slightly lower than with Fluazifop. Glufosinate, by contrast, provided near-complete control of both hybrids and parent by 31 DAA Control at very high levels continued through 42 DAA.

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[10] Roundup PRO is a registered trademark of Monsanto Technology, LLC
[11] Teejet is a trademark of Spraying Systems Company

Glyphosate provided only marginal and commercially unacceptable control of susceptible *A. capillaris* at 45 DAA in the fall experiment (Table 11). It provided little activity on any of the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop also provided marginal and commercially unacceptable control of both hybrids and parent by 45 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim provided similar activity as fluazifop on *A. capillaris* hybrids and parents by 45 DAA although control tended to be slightly lower than with Fluazifop. Glufosinate, by contrast, provided near-complete control of both hybrids and parent by 31 DAA Control at very high levels continued through 42 DAA.

Glyphosate provided only marginal and commercially unacceptable control of susceptible *A. capillaris* at 45 DAA in the fall experiment (Table 12). Glyphosate provided little activity on the hybrid accessions of *A. idahoensis* indicating presence of the *cp4 epsps* gene. Fluazifop also provided poor control of both hybrids and parent by 45 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim provided similar control to fluazifop on *A. idahoensis* hybrids and parents by 42 DAA. Glufosinate provided near-complete control of both hybrids and parents by 31 DAA and control was maintained at a high level through 42 DAA.

Glyphosate provided only marginal and commercially unacceptable control of susceptible *A. pallens* at 45 DAA in the fall experiment (Table 13). Glyphosate provided little activity on the hybrid accession of *A. pallens* indicating presence of the *cp4 epsps* gene. Parent germplasm of this species was unavailable for this experiment. Fluazifop failed to provide commercial control of the hybrids or parents by 45 DAA. Typically the effects were slower to develop than with glyphosate. Sethoxydim performed similarly to fluazifop for control of *A. pallens* hybrids by 45 DAA although control tended to be slightly lower than with Fluazifop. Glufosinate provided near-complete burndown of both hybrids and parent by 31 DAA and this high level of control was maintained through 45 DAA.

Glyphosate provided no activity on either of the hybrid accession of *A. pallida* indicating presence of the *cp4 epsps* gene (Table 14). Parent germplasm of this species was unavailable for this experiment. Fluazifop provided marginal, commercially unacceptable control of the hybrids by 45 DAA. Sethoxydim was no better than fluazifop control of *A. pallida* hybrids by 42. Glufosinate provided excellent control of these hybrids at 28 - 42 DAA.

Glyphosate failed to control of susceptible *P. monspeliensis* at 45 DAA (Table 15). Not surprisingly, it provided little activity on either of the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop provided commercial control of the hybrid accessions and parent by 45 DAA. Sethoxydim was equally effective in control of *P. monspeliensis* hybrids and parent by 45 DAA. Glufosinate provided near-complete burndown of both hybrids and by 31 DAA and control persisted through 45 DAA evaluations.

Glyphosate provided unacceptable control of susceptible *P. fugax* at 14-42 DAA (Table 16). It provided no activity on the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop provided near-commercial control of the hybrid and parent by 45 DAA. Sethoxydim provided effective control of the *P. fugax* hybrid by 45 DAA. Control of the parents was less consistent. Glufosinate provided almost perfect control of the hybrids and parents at 31- 45 DAA.

Glyphosate provided unacceptable control of susceptible *P. viridis* at 45 DAA (Table 17). It provided little activity on the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop provided near commercial control of the hybrids and very effective control of the parent by 45 DAA. Sethoxydim was equivalent performance to fluazifop of the *P. viridis* hybrids and parents by 45 DAA. Glufosinate provided excellent control of the hybrids and parents by 28 DAA and control was maintained through 45 DAA.

Glyphosate provided unacceptable control of susceptible *A. trinii* at 45 DAA (Table 18). It provided little activity on the hybrid accessions indicating presence of the *cp4 epsps* gene. Fluazifop was variable in control of both hybrids and parents at 45 DAA. Control with sethoxydim was similar to fluazifop on *A. trinii* hybrids and parents at 45 DAA. Glufosinate provided near-complete burndown of hybrids and parents by 31 DAA. The high level of control with glufosinate observed at 31 DAA persisted through 45 DAA evaluations with both hybrids and parents.

Glyphosate provided complete control of susceptible commercial creeping bentgrass cultivars at 31-45 DAA (Table 19). Neither fluazifop and nor sethoxydim provided a high level of control of commercial cultivars by 45 DAA. The effects that were observed were slower to develop than with glyphosate. Glufosinate also provided complete control of commercial cultivars at 31 - 45 DAA.

Glyphosate provided complete control of susceptible *A. castellana* at 31-45 DAA (Table 20). Neither fluazifop and nor sethoxydim provided commercial control of *A. castellana* by 45 DAA. The effects that were observed were slower to develop than with glyphosate. Glufosinate also provided complete control of these susceptible accessions at 31 - 45 DAA.

Glyphosate provided unacceptable control of susceptible *A. canina* at 45 DAA (Table 21). Fluazifop was variable in control at 45 DAA. Control with sethoxydim was inferior to fluazifop on *A. canina* at 45 DAA. Glufosinate provided near-complete control of two of the three accessions by 31 DAA. The high level of control with glufosinate observed at 31 DAA persisted through 45 DAA evaluations with these two accessions.

Conclusions

Results from the fall, 2002 experiments were different from previous work in a very significant way. The spray date was much later than with previous trials on either hybrids or *A. stolonifera.* This may have affected the performance of both glyphosate and the ACCase inhibitors that are hampered by conditions of low temperature and low

light. These conditions are especially typical of Western Oregon where this experiment was conducted. A fast-acting active ingredient such as glufosinate clearly benefits from these end-of-season conditions of which the results of this experiment provide confirmation. Clearly, the ACCase inhibitor herbicides can compete with glyphosate under these environmental conditions however neither offers a compelling solution during this time of year. Glufosinate would be the solution of choice when removal is required late in the season. The potential for a sequential application of the ACCase inhibitor class would seem to be obviated by the onset of winter.

Summary

Effective herbicide solutions were identified in these experiments to address the need to control hybrids that may form on rare occasion from pollen flow to sexually compatible relatives from Roundup Ready creeping bentgrass. The solution of choice may differ depending on environmental conditions characteristic of seasonal variation. Multiple active ingredients are available (ACCase inhibitor herbicides or glufosinate) that via either single or multiple applications can remove these plants as effectively as glyphosate can remove their non-transgenic predecessors.

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Table 1. Response of *A. gigantea* hybrids and parent as individual plants to postemergence herbicides, spring, 2002.

		----------Control 0-5 Scale----------														
		----------RR 1----------.....					----------RR 2----------					----------RS----				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	0	0	0	0	0	0	0	0	0	0	0.9	3.1	4.9	5	0
Glufosinate	1.6	3	2.7	5	3.2	16.5	4	4.1	5	4	10	2.4	3.1	4.8	3.9	6.2
Fluazifop	0.4	1	1.8	3.6	4.7	0	1.4	4.7	4.1	4.9	0	0.9	2	3.6	4.6	0
Sethoxydim	0.6	0	2	3.6	4.6	0	0	2.3	4.1	4.7	0	0	3	3.2	4.2	0
Check		0	0	0	0	0	0	0	0	0	0	1.3	0	0	0	0

Table 2. Response of *A. idahoensis* hybrids as individual plants to postemergence herbicides, spring, 2002.

		----------Control 0-5 Scale----------									
		----------RR 1----------					----------RR 2----------				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	0	0	0	0	0	0.3	0	0	0	0
Glufosinate	1.6	3.4	4	5	5	0	3	4	5	4.9	0
Fluazifop	0.4	1	2.2	4.6	5	0	1	17	3.7	4.9	0
Sethoxydim	0.6	0	2.3	4.2	4.8	0	0	2.1	3.7	4.6	0
Check		0	0	0	0	0	0	0	0	0	0

Table 3. Response of *A. pallens* hybrids and parent as individual plants to postemergence herbicides, spring, 2002.

		----- Control 0-5 Scale------				
		----------RR----------				
Treatment	Rate kg/ha	7	14	28	42	42 RG
Glyphosate	1.6	0.3	0.3	0	0	0
Glufosinate	1.6	3	4	5	4.5	3.7
Fluazifop	0.4	1.3	2.3	4.1	4.8	0
Sethoxydim	0.6	0	2.5	4	4.7	0
Check		0	0	0	0	0

Table 4. Response of *P. monspeliensis* hybrids and parent as individual plants to postemergence herbicides, spring 2002.

		Control 0-5 Scale														
		RR 1					RR 2					RS				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	0.7	0.7	0	0	0	0.3	0.3	0	0.7	0	2.8	5	5	5	0
Glufosinate	1.6	3.6	4	4.9	4	7.3	3.3	4	5	4.5	2.8	3.8	4	5	5	0
Fluazifop	0.4	1.1	2.2	4	4.9	0	1.7	2.8	4.7	5	0	1.9	3.6	4.8	5	0
Sethoxydim	0.6	0	2.7	4.1	5	0	0	3	5	5	0	0	4.3	5	5	0
Check		0	0	0	0	0	0	0	0	0.7	0	1.3	0	0	2.3	0

Table 5. Response of *P. fugax* hybrids as individual plants to postemergence herbicides, spring, 2002.

		Control 0-5 Scale									
		RR					RS				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	0	1.3	0	0	0	2.3	5	5	5	0
Glufosinate	1.6	3.1	3.5	5	4.1	3.1	3.2	4	4.8	4.8	3.1
Fluazifop	0.4	1.3	2.7	4.3	5	0	1.1	3.3	5	5	0
Sethoxydim	0.6	0	2	3.9	5	0	0	3.1	4.9	5	0
Check		0	0	0	0	0	0	0	0	0	0

Table 6. Response of *P. viridis* hybrids and parent as individual plants to postemergence herbicides, spring, 2002.

		Control 0-5 Scale									
		RR					RS				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	0	0	0	0	0	2.7	4.3	5	5	0
Glufosinate	1.6	3.3	4	5	4.2	7.3	3.5	4	4.8	5	0
Fluazifop	0.4	1.1	2.5	3.5	4.7	0	1	3.2	4.8	5	0
Sethoxydim	0.6	0	2.5	3.8	4.7	0	0	3.7	4.8	5	0
Check		0	0	0	0	0	0	0	0	0	0

Table 7. Response of *A. capillaris* hybrids and parents as individual plants to postemergence herbicides, spring, 2002.

		Control 0-5 Scale																			
		RR 1					RR 2					RS 1					RS 2				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	0.2	0	0	0	0	0.3	0	1.7	0	0	1.3	4.3	5	5	0	1.7	4.7	5	5	0
Glufosinate	1.6	3.4	3.9	5	4.5	2.4	3.4	4	4.9	4.5	3.4	3.5	4	5	4.8	0.7	3	4	5	5	0
Fluazifop	0.4	1	2.2	4.2	4.9	0	1	2.2	4.5	4.9	0	1	1.7	3.8	4.8	0	0.7	1.7	4.2	5	0
Sethoxydim	0.6	0	2.3	3.4	4.4	0	0	2.3	4.1	4.9	0	0	2	3.2	4.3	0	0	2.4	3	4.4	0
Check		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		Control 0-5 Scale				
		RR 3				
Treatment	Rate kg/ha	7	14	28	42	42 RG
Glyphosate	1.6	0	0	0	0	0
Glufosinate	1.6	3.4	4	5	4.8	1.9
Fluazifop	0.4	1.2	1.9	4.4	4.9	0
Sethoxydim	0.6	0	2.1	3.3	4.3	0
Check		0	0	0	0	0

Table 8. Response of commercial cultivars of creeping bentgrass as individual plants to postemergence herbicides, spring, 2002.

		Control 0-5 Scale														
		Southshore					Crenshaw					Penneagle 1				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	2.4	4.1	5	5	0	1.7	4.3	5	5	0	2.7	4.7	5	5	0
Glufosinate	1.6	3.1	3.8	5	4.5	3.5	2.8	4	5	4.8	2.7	3.3	4	5	4.3	8.3
Fluazifop	0.4	1.2	2.1	3.9	4.9	0	1	2	4	4.8	0	1	2	4.3	4.7	0
Sethoxydim	0.6	0	1.6	3.7	4.8	0	0	2.3	4.3	4.8	0	0	2.3	4	5	0
Check		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		Control 0-5 Scale														
		A-4					Backspin					Penneagle 2				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	2	4.3	5	5	0	1.7	4.5	5	5	0	2	4.2	5	5	0
Glufosinate	1.6	3.1	4	4.9	4.1	6.1	3.5	4	5	4.3	6.8	3.3	4	5	4.9	4.2
Fluazifop	0.4	1.2	2	3.8	4.7	0	1.1	2.2	4.4	4.9	0	1.6	2.9	4.6	4.9	0
Sethoxydim	0.6	0	2.2	3.4	4.7	0	0	2.5	3.7	4.8	0	0	2.7	4.6	4.9	0
Check		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 9. Response of ASR 368 as individual plants to postemergence herbicides, spring, 2002.

		Control 0-5 Scale									
		ASR 368 1					ASR 368 2				
Treatment	Rate kg/ha	7	14	28	42	42 RG	7	14	28	42	42 RG
Glyphosate	1.6	0.2	0	0	0	0	0.2	0	0	0	0
Glufosinate	1.6	3.3	4	5	4.9	7.3	3.3	4	5	4.9	0
Fluazifop	0.4	1.3	2.6	4.7	5	0	1.1	2.3	4.6	5	0
Sethoxydim	0.6	0	2.2	4.7	5	0	0	2.6	4.3	4.9	0
Check		0	0	0	0	0	0	0	0	0.2	0

Table 10. Response of *A. gigantea* hybrids and parents as individual plants to postemergence herbicides, Fall, 2002.

		Control 0-5 Scale																				
		RR 1			RR 2			RR 3			RR 4			RS 1			RS 2			RS 3		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0	0.3	0	0.3	0.3	0	0	0.7	0.3	0	0.7	0	2	2.7	3.3	2.3	3	3.3	2.5	3.5	5
Glufosinate	1.6	4	5	5	4	5	5	4.3	5	5	3.4	3.5	4	4.3	5	5	4.3	5	5	4.5	5	5
Fluazifop	0.4	0.3	2	3	0.3	3.7	0.3	3	4	0.3	2.7	4.3	1.7	0.7	2.7	4.3	0.7	2	4	1	2.5	4
Sethoxydim	0.6	0	2.7	3.7	0.3	2.3	3.7	0.3	2.7	3.3	0.3	2.7	3	0.7	2	3.7	0.7	2.7	3.3	0.5	2	3
Check		0	0	0	0	0	0	0	0.3	0	0	0	0	1	1.7	1.7	1	1.7	1.7	0	0.5	0.5

		Control 0-5 Scale								
		RS 4			RS 5			RS 6		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	2.7	2.3	4	2.3	2.7	4	1.7	2.7	3.3
Glufosinate	1.6	4.7	5	5	4.3	5	5	4.3	5	5
Fluazifop	0.4	1	3.7	4.3	1.7	3.3	4.3	0.3	2	3.7
Sethoxydim	0.6	1	2.7	4	1	2.7	3.7	0	1.7	3.3
Check		2	1.7	3.3	1.7	1.7	3.3	0.3	1.7	1.7

Table 11. Response of *A. capillaris* hybrids and parents as individual plants to postemergence herbicides, fall, 2002.

		----------Control 0-5 Scale----------																	
		-----RR 1-----			---- RR 2-----			-----RR 3------			------RS 1-----			-----RS 2-----			-----RS 3------		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0.3	0.3	0	0.7	0.7	0.3	0.3	0.3	0	1	2.7	3.3	1.3	2.7	3.3	1.3	2.7	3.3
Glufosinate	1.6	5	5	5	4.3	5	5	4.7	5	5	4.3	5	5	4.3	5	5	4.5	5	5
Fluazifop	0.4	1	2.3	3.7	1	3	4	0.3	2.3	3.7	0.7	1.3	3.3	0.3	2	2.7	0	2	3.3
Sethoxydim	0.6	0.7	3	3.7	0.7	3	3.3	0.7	2.3	3.7	0.3	2	3.3	0.3	2.3	3	0	1.7	2
Check		0	0	0	0	0	0	0	0	0	0.7	1.3	1.7	0.7	1.3	1.7	0.7	1.3	1.7

Table 12. Response of *A. idahoensis* hybrids and parents as individual plants to postemergence herbicides., fall, 2002

		----------Control 0-5 Scale----------																	
		-----RR 1-----			---- RR 2-----			-----RR 3------			------RS 1-----			-----RS 2-----			-----RS 3------		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0.3	0.7	0.3	0.7	0.7	1.3	0.3	0.3	0	1.3	3	3.3	1.7	3.3	3.3	1.3	3	3.3
Glufosinate	1.6	4.7	5	5	5	5	5	4.7	5	5	4	5	5	4.3	5	5	4.3	5	5
Fluazifop	0.4	0.3	2.3	4	0.7	3.3	4	0.3	2.3	3.7	0	1.3	2	0	1.3	2.3	0	1	2.3
Sethoxydim	0.6	0.3	3	3.3	0.7	2.3	3.3	0.7	2.7	4	0	1.3	2	0	1.3	2.3	0	1	2.3
Check		0	0.3	1	0	0.3	0.7	0.3	0.7	2	0.7	1.3	1.7	0.7	1.7	1.7	0.3	1.3	1.7

Table 13. Response of *A. pallens* hybrids and parents as individual plants to postemergence herbicides, fall, 2002.

		Control 0-5 Scale																				
		RR 1			RR 2			RR 3			RR 4			RS 1			RS 2			RS 3		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0.7	0.7	0	0.7	0.7	0	0.3	0.7	0	0.3	0.7	0	1	2.7	3.3	1	2.7	3.3	1.3	3	3.3
Glufosinate	1.6	4.7	3.7	5	5	5	5	4.3	5	5	4	5	5	4.7	5	5	4.3	5	5	4	5	5
Fluazifop	0.4	0.3	2.7	4	0.7	3.3	4.7	0.3	3	4.3	0.3	3.3	3.7	0	3	3.7	0.3	3	3.3	0.3	2.7	3.7
Sethoxydim	0.6	0	3.7	3.7	0.3	2.7	3.7	0.3	3	4	0.7	3	4.3	0.3	2	3.3	0.3	2	3	0.3	2.3	3
Check		0.3	0	0	0.3	0	0	0.3	0.3	0	0	0.3	0	0.3	1.3	1.7	0.3	1.3	1.7	0.3	1.7	1.7

Table 14. Response of *A. pallida* hybrids as individual plants to postemergence herbicides, fall, 2002.

		Control 0-5 Scale											
		RR 1			RR 2			RR 3			RR 4		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0.3	0.3	0	0.3	0.3	0	0	0.3	0	0.3	0.7	0
Glufosinate	1.6	4.3	5	5	4.7	5	5	4.3	5	5	4.7	5	5
Fluazifop	0.4	0.7	2.3	3.3	0.7	2	3.7	0.7	2.3	4	0.3	2.3	3.7
Sethoxydim	0.6	0.7	2.7	3.7	0.3	2	3.7	0.3	2.3	4	0	2.3	3.7
Check		0	0	0	0.3	0	0	0	0	0	0	0	0

Table 15. Response of *P. monspeliensis* hybrids and parents as individual plants to postemergence herbicides, fall, 2002.

		----------Control 0-5 Scale----------											
		-----RR 1-----			---- RR 2-----			-----RR 3------			------RS 1-----		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0.7	0.3	0	1	1	0.7	1	0.7	0	2.7	3.3	3.5
Glufosinate	1.6	4.7	5	5	4.7	5	5	5	5	5	4.3	5	5
Fluazifop	0.4	1.3	3	4.3	1.7	3	4.7	1.7	3.3	4.3	1.7	4	4.7
Sethoxydim	0.6	1.3	3.7	4.3	1	2.3	4.7	0.7	3	4.7	0.4	3.3	4.5
Check		0.3	0	0	0.3	0	0	0.3	0	0	0	0	0

Table 16. Response of *P. fugax* hybrids and parents as individual plants to postemergence herbicides, Fall, 2002.

		----------Control 0-5 Scale----------																	
		-----RR 1-----			---- RR 2-----			-----RR 3------			------RS 1-----			-----RS 2-----			-----RS 3------		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0	0	0	0.7	0	0	0.3	0	0	2	3.3	3.3	2.3	3.3	3.3	1.5	2.3	2.3
Glufosinate	1.6	4.3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Fluazifop	0.4	1.3	3	4	1.3	3	4	1.7	3	4	0.7	4	5	0.7	3.7	5	0.7	4	4.7
Sethoxydim	0.6	0.7	3.3	4.7	0.7	3.7	4.7	1	3.3	4.7	1.3	3.7	5	1	3.7	5	0.7	3	5
Check		0	0	0	0	0	0	0	0	0	1	1.7	2	1	1.7	1.7	1	2	1.7

Table 17. Response of *P. viridis* hybrids and parents as individual plants to postemergence herbicides, fall, 2002.

		Control 0-5 Scale																	
		RR 1			RR 2			RR 3			RS 1			RS 2			RS 3		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0.3	0.3	0	0.3	0.3	0	0.3	0	0	2.7	3.7	4	2.3	3.7	3.7	3.3	3.7	5
Glufosinate	1.6	4.7	5	5	4.7	5	5	4.7	5	5	5	5	5	4.7	5	5	5	5	5
Fluazifop	0.4	0.7	3.3	4	0.3	3.7	4	1	3.3	4	1.7	4	5	1.3	3.7	5	1.3	3.7	4.7
Sethoxydim	0.6	2	3.3	4.3	0.7	3.7	3.7	0.7	3.7	4	1	3.7	5	0.7	4	5	0.7	4	5
Check		0	0	0	0	0	0	0	0	0	1.3	2	1.7	1.3	2	1.7	1.3	2	1.7

Table 18. Response of *A. trinii* hybrids and parents as individual plants to postemergence herbicides, fall, 2002.

		Control 0-5 Scale																				
		RR 1			RR 2			RR 3			RR 4			RS 1			RS 2			RS 3		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	0.7	0.7	0.7	1	0.3	0.3	0.7	0.7	0.3	0.7	0.7	0	1.3	2.7	3.7	2	2.7	3.7	1.7	2.7	3.7
Glufosinate	1.6	4	5	5	4.4	5	5	4.7	5	5	4.7	5	5	4.7	5	5	4	5	5	4	5	5
Fluazifop	0.4	1	3	4.3	1	2.7	3.3	1	3	4	0.7	3	4	0.3	1.7	3.7	0.3	1.7	3.3	0	1.7	2.7
Sethoxydim	0.6	0.7	2.3	4	0.7	2	3.3	0.7	2.7	4	1.3	3	4.3	0	2	3	0.3	2	3.3	0.3	2	3.3
Check		0	0.3	0	0.3	0.7	0	0.7	0	0	0.3	0	1.3	0.3	1.7	2	0.7	1.7	2.3	0.7	1.3	2.3

Table 19. Response of commercial cultivars as individual plants to postemergence herbicides, fall, 2002.

		Control 0-5 Scale																	
		Seaside			Southshore			Penncross			L-93			palustris 1			palustris 2		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	3	5	5	2	4	5	2.5	5	5	2	5	5	1.5	4	5	2.5	4.5	5
Glufosinate	1.6	4.5	5	5	4.5	5	5	4.5	5	5	4.5	5	5	4.5	5	5	4.5	5	5
Fluazifop	0.4	1	3	3.5	0.5	2.5	3	1.5	4	4	1	3.5	3.5	0.5	2	2.5	0.5	4	4
Sethoxydim	0.6	0.5	2.5	3	0	3	4	0.5	3	4	0.5	2.5	3.5	1	3	3.5	0.5	3	4
Check		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 20. Response of susceptible *A. castellana* as individual plants to postemergence herbicides, fall, 2002.

		Control 0-5 Scale																	
		RS 1			RS 2			RS 3			RS 4			RS 5			RS 6		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	2	4.5	5	2	4.5	5	2	5	5	1	4	5	1	4.5	5	2	5	5
Glufosinate	1.6	4.5	5	5	4.5	5	5	4	5	5	4.5	5	5	4.5	5	5	4.5	5	5
Fluazifop	0.4	1	3	4	1.5	3	3.5	1.5	2.5	4	0.5	2	3	0.5	2.5	3.5	0.5	2.5	4
Sethoxydim	0.6	1	3	3.5	1	3	3.5	1	2.5	4	0.5	2	3	0.5	3.5	3.5	0.5	2.5	3
Check		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7

Table 21. Response of susceptible *A. canina* as individual plants to postemergence herbicides, fall, 2002.

		----------Control 0-5 Scale----------								
		-----RS 1-----			---- RS 2-----			-----RS 3------		
Treatment	Rate kg/ha	16	31	45	16	31	45	16	31	45
Glyphosate	1.6	1.3	3	3.3	0.3	1.7	1.7	1	2.7	3.3
Glufosinate	1.6	4.3	5	5	4.3	3.3	3.3	4.3	5	5
Fluazifop	0.4	0	2	3	0.3	2.3	3.7	1.3	2.3	4
Sethoxydim	0.6	0.3	1	3.3	0	1	2.3	0.3	0.7	2.7
Check		0.7	1.3	1.7	0.7	1.3	1.7	0.3	1.7	0

Appendix VIII

Supporting Information

United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

4700 River Road
Riverdale, MD 20737

October 21, 2003

Mr. Terry Stone
Director, Biotechnology Regulatory Affairs
The Scotts Company
14111 Scottslawn Road
Marysville, OH 43041

Dear Mr. Stone:

The submitted revised petition (03-104-01p) has provided substantial data and information to show that the creeping bentgrass (*Agrostis stolonifera*) line genetically engineered to be resistant to glyphosate is not otherwise weedier than its parents. APHIS/BRS is not aware of other experiments to perform that might substantially further our understanding as to any unexpected weediness characteristics of the transformed bentgrass relative to its parents and other creeping bentgrass cultivars.

However, there are many matters to consider for a robust perennial grass species with an extensive distribution and many habitats, in releasing the plants containing a gene inserted to confer resistance to a major herbicide. Since APHIS/BRS deems additional input to be necessary in order to better gauge the risks, we will solicit information and comments through the publication of a *Federal Register* notice and the convening of a scientific workshop on the subject. It is our intent to deem the petition "complete" and make it available in electronic form to the public. Before doing so, we are giving you this opportunity to respond to points in the petition where we have some particular concern (see below). We are leaving it up to you whether or how to respond, perhaps by altering the text of the petition or through a separate document to APHIS.

p. 31 line 9 and similarly p. 65 line 36. "The formation of hybrids with related species is rare…" Rare implies that creeping bentgrass is unlikely to cross with its relatives. However, there are many examples of spontaneous hybridization, with reports beginning at least 150 years ago; for prominent recent views, see the information in Warnke (2003) which ("…would seem to indicate that gene flow between species is highly likely"), and in Brede and Sellmann (2003) ("…a number of *Agrostis* species have been shown to intercross, some resulting in viable offspring. In a survey…in Finland …some interspecific 'hybrids are rather common [and] in some areas they even form an important part of the vegetation.'") These chapters are in Casler and Duncan (2003), *Turfgrass Biology, Genetics, and Breeding*. The F_1 hybrids of *A. stolonifera* and *A. capillaris* (colonial bentgrass) can be plentiful and apparently long-lived in intermediate areas between the parental habitats (e.g., Forde 1991; Widén 1971; Bradshaw 1958, 1959 – references in petition). In New Zealand, Forde (1991) found these hybrids to be vigorous but sterile or of low fertility (although with 41% pollen viability), whereas the hybrids of *A. capillaris* and *A. castellana* (dryland bentgrass)

"proved to be nearly as fertile as the parents", with considerable introgression having occurred into *A. capillaris*. Thus APHIS/BRS feels that movement of the resistance gene into other *Agrostis* species is a very likely possibility should the glyphosate-resistant (Roundup Ready) creeping bentgrass establish outside of heavily mown sites.

Forde, M.B. 1991. Assessment of the extent and taxonomic significance of hybridism in naturalised bent grasses. *New Zealand Journal of Botany* 29: 158-161.

p. 31 line 12. "the species neither exhibits characteristics of a weed nor has a history of weediness." While not regarded as a major weed in the U.S., *Agrostis stolonifera* has some weedy characteristics (e.g. Baker 1974), which include the ability to spread vegetatively, rapid growth through vegetative phase to flowering, being cross-pollinated and wind-pollinated, high seed output in favorable environments, adaptations for short- and long-distance dispersal, and competitive growth in disturbed areas. It is for example included in Agriculture and Agri-Food Canada's *Inventory of Canadian Agricultural Weeds* (Darbyshire 2003), BASF's *Grass Weeds in World Agriculture* (Behrendt and Hanf 1979), and Ciba-Geigy's *Grass Weeds* (Häfliger and Scholz 1981) — occurring in all five habitats that they score (rotation crops, perennial crops, grassland, aquatic biotopes, and waste places).

Baker, H.G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5: 1-24.

p. 56 line 33. "The economic perspective implies that while *A. stolonifera* may be undesirable in certain locations, only host sites with sufficient economic or social value will warrant the expense and/or effort to manage it as an economic pest. In this context, *A. stolonifera* may be considered an economic weed in a limited number of circumstances:" We recognize that in the primary context of the golf course with its intensive *Creeping Bentgrass Management* (Dernoeden 2000), "When properly irrigated and otherwise managed, creeping bentgrass becomes a very tough and resilient grass." But the four circumstances sketched in the petition (p. 56) seem all encompassing and therefore the possibilities of *A. stolonifera* as a weed could appear unlimited! You may wish to clarify your probable meaning to focus on contexts where glyphosate or another means of controlling creeping bentgrass might be or become an actual concern.

p. 58 line 27. "*A. stolonifera* develops at a slower rate than other grasses and requires an earlier fall planting date than most other turfgrass species." The majority of the petition's seed plantings for seed establishment and flowering experiments were done in October; you may wish to explain whether this was past optimal planting for the northern sites.

That sentence could in error be lifted out of the turf seed production context, whereas creeping bentgrass is known to have a fairly high maximum potential relative growth rate (1.48 g/g per week), for example below RGR_{max} of buckhorn plantain (*Plantago lanceolata*), slightly higher than in rough bluegrass (*Poa trivialis*) and well above the RGR_{max} of white clover (*Trifolium repens*) and common dandelion (*Taraxacum officinale*) (Grime and Hunt 1975).

Grime, J.P., and R. Hunt. 1975. Relative growth-rate: Its range and adaptive significance in a local flora. *Journal of Ecology* 63: 393-422.

p. 58 line 39. "*A. stolonifera* is a shallow-rooted species that requires more consistent soil moisture than other species in order to persist and produce seed. Summers are very dry in the Willamette Valley... Other grasses typically are produced without irrigation, so *A. stolonifera* do not thrive in their midst." These statements could be construed to imply that *A. stolonifera* will not be able to establish and produce seed outside of managed, irrigated sites. However,

research by Mueller-Warrant et al. (2002) in text and a detailed map reports that *Agrostis* spp. are weeds "widely distributed in the Willamette Valley, although the northwest section is relatively free," and the *"Guide for Using Willamette Valley Native Plants Along Your Stream"* (1998) lists *A. stolonifera* (and *A. palustris*), as well as *A. capillaris*, *A. castellana* and *A. gigantea* (redtop), among "invasive non—native plant species" that are not recommended for planting in or near streams or wetlands "due to their aggressive growth habit and competitiveness. They can take over and dominate native plant species." Information in a 2001 letter submitted to APHIS (by M. Jordan for The Nature Conservancy) has more detail about *A. capillaris* as an invasive in the Willamette Valley in prairie communities. Thus in contrast to the petition statements, it appears that *A. stolonifera* has naturalized widely in the Willamette Valley outside of production sites, in the absence of irrigation.

"Guide for Using Willamette Valley Native Plants Along Your Stream." 1998. Edited by South Santiam Watershed Council, [USDA] Natural Resources Conservation Service, and Linn Soil and Water Conservation District. 25 pp.

Jordan, M. 12/2001. [Letter to J. White, USDA/APHIS, on potential commercial release of *Agrostis stolonifera* and *Poa pratensis* genetically altered for resistance to herbicides.] Stewardship Ecologist, The Nature Conservancy, Long Island and South Fork/Shelter Island Chapters, Cold Spring Harbor, New York. 1 + 5 pp.

Mueller-Warrant, G.W., L.R. Schweitzer, R.L. Cook and A.E. Garay. 2002. Geographic distribution of prominent weeds of grass seed production. *"2002 Seed Production Research at Oregon State University, USDA-ARS Cooperating*", edited by W.C. Young III. http://cropandsoil.oregonstate.edu/seed-ext/Pub/2002/36.html

p. 64 line 4. "…the most likely means of introduction in a natural system outside of the geographic origin of *A. stolonifera* is through commercial seed... Stolon introduction could occur as a stowaway on tillage or excavating equipment but is highly unlikely in an isolated ecosystem. There are no reports of ecosystem disturbance by *A. stolonifera* outside of these conditions."

It is difficult to reconcile those statements with information like the following. "Plants from ditches, streambanks, and wet spots in pastures develop long stolons…and soft green blades… These plants are completely naturalized [in Ontario] and have spread rapidly by vegetative and seed means and now often form continuous mats along secluded shores where they survive periodic submergence" (Dore and McNeill 1980). "Introduced, scattered nearly throughout Missouri, but apparently absent from some southern portions of the Ozark Division (native of Europe, cultivated and naturalized nearly throughout the U.S. and Canada)" (Yatskievych 1999). This species has been reported spontaneously present and is so mapped by county per state (Kartesz 2003), being known to occur in the majority of the counties in the U.S. (including Hawaii), except for the warmer southern portions of the states in the Southeast. After extensive fieldwork in Fennoscandia, followed by intensive analysis, Widén (1971) determined that several different *Agrostis* F_1 hybrids were significant components of the vegetation in some areas, probably from vegetative dispersal along rivers. In California's Central Coast and South Coast regions, *A. stolonifera* is listed as a moderate threat to native species or ecosystems in riparian areas and wetlands (Dudley 1998). Similar strong invasiveness as the petition noted on Marion Island has occurred on other sub-Antarctic islands and on Gough Island (Frenot et al. 2001; Jones et al. 2003).

Dore, W.G., and J. McNeill. 1980. *Grasses of Ontario*. *Agrostis* L. (pp. 288-298 & plate 40). Agriculture Canada Monograph No. 26.

Dudley, T. 1998. Exotic plant invasions in California riparian areas and wetlands. *Fremontia* 26(4): 24-29.

Frenot, Y., J.C. Gloaguen, L. Massé and M. Lebouvier. 2001. Human activities, ecosystem disturbance and plant invasions in subantarctic Crozet, Kerguelen and Amsterdam islands. *Biological Conservation* 101: 33-50.

Jones, A.G., S.L. Chown, P.G. Ryan, N.J.M. Gremmen and K.J. Gaston. 2003. A review of conservation threats on Gough Island: A case study for terrestrial conservation in the Southern Oceans. *Biological Conservation* 113: 75-87.

Kartesz, J.T. 2003. *A Synonymized Checklist and Atlas with Biological Attributes for the Vascular Flora of the United States, Canada, and Greenland*, 2nd Ed. In J.T. Kartesz and C.A. Meacham, *Synthesis of the North American Flora*, Version 1.985 (ms.). BONAP, University of North Carolina, Chapel Hill, and Jepson Herbarium, University of California, Berkeley.

Yatskievych, G. 1999. *Steyermark's Flora of Missouri*, Rev. Ed., Vol. 1. *Agrostis* L. (bent grass) (pp. 623-629). Missouri Dept. of Conservation, Jefferson City, and Missouri Botanical Garden Press, St. Louis.

p. 127 lines 28 and 32. R1 and F1 are defined as the second progeny population. It seems necessary to explain: What is the first progeny population?

p. 127 line 36. F2. You may wish to make clear whether the pollen from F1 ASR368 derived from a single plant or a population of plants.

pp. 136-138 Tables VI.A.3-5. Survivability does not seem to be what was actually measured. The assays are based on plant counts and do not distinguish late germination from survivability. In some cases no seedlings survived perhaps especially because of the late planting date. You may wish to clarify why more optimal conditions were not used for the assays.

pp. 138-139 Tables VI.A.6-8. These tables are used to show that the creeping bentgrass did not establish well in competitive and non-competitive environments. However the late seeding date may be the main reason for the lack of germination and establishment. You may wish to clarify why the tests were conducted so late in the season.

p. 142 line 6. "Seed establishment for the Spring 2002 irrigated and non-irrigated plantings was essentially zero …" Why should the overall survivability be so low even in the irrigated bare-soil plots? On the whole, APHIS accepts the petition's conclusion that there is not a difference in seed establishment between ASR368 and commercial turf varieties. Although the overall experimental difficulty in establishing any of the creeping bentgrass varieties even under adequate moisture conditions supports the view that creeping bentgrass cannot spread very easily by seed, it is contradicted by the observation and experience that golf greens can be established by seeding. APHIS has the concern that these experiments were done under such suboptimal conditions that the typical competitive ability of the bentgrasses was not observed.

pp. 150-151 Tables VI.B.1-2 show the percent of nodes producing tillers. Table VI.B.1 indicates that Roundup Ready (RR) ASR368 plants produce more tillers from nodes than do the glyphosate-susceptible (or -sensitive) (RS) ASR368 plants and four commercial cultivars, and that the RS plants produce more tillers than the cultivar Crenshaw. The follow-up

experiment (TableVI.B.2) surprisingly does not include data from the susceptible plants, the cultivar Crenshaw, nor in fact any of the comparators in the first experiment. Hence the significant difference observed in the first experiment is apparently not adequately pursued in the second experiment, which analyzes F1 progeny. The background of the ASR368-A and ASR368-B progeny may need clarification (p. 149). Were these progeny derived from a single cross, and if so what criteria were used to select the maternal parents for that cross? On page 157 in section VI.B.3 second paragraph line 5, the petition concludes that statistical differences between RR and RS did not occur in the second experiment, but no data are given for the RS in Table VI.B.2. APHIS believes that the most informative comparators in this experiment are the F1 progeny segregating as susceptible (RS) (p. 127). Inclusion of data from these susceptible null segregants would have greatly improved the meaning of the second experiment.

pp. 153-157 Tables VI.B.3-10. In tables 3-7 the number of nodes sprouting tillers appears to be cumulative, but in tables 8-10 the presentation appears to be the incremental increase per time interval. You may want to clarify. Why change the presentation format?

pp. 171-173 Tables VI.C.13-16 (on mean plant diameter). You may want to clarify whether the data are for RR, RS, or a mixture of both, and whether independent data were available for both RR and RS.

pp. 197-198 Tables VI.D.13-14 and pp. 214-216 Tables VI .F.5-9, which present data for the RR and RS segregating populations, are compelling in dispelling the possibility that the random insertion of the glyphosate-resistance gene has affected yield and flowering characteristics of this biotechnologically derived *A. stolonifera*.

p. 256 line 35. "This area has been the predominant creeping bentgrass seed production location for the past 75 years (Schoth, 1930)." You may want to reference a recent article characterizing the production.

p. 260 line 8. "Three replications, each containing 20 plants of each seedling lot". You may want to clarify whether these are 20 clones or 20 genetically distinct seedlings.

pp. 263-264 Tables VII.C.1a-b and C.2. All data on percentage RR plants and percentage hybrids apparently would need to be multiplied by 2 to reflect that only 50% of the pollen from the glyphosate-resistant (RR) plants carry the resistance gene.

p. 267 Table VII.C.6. It is not clear what "Ground Cover (%)" means. How can it exceed 100%? You may want to elaborate (p. 260) on how it was calculated and measured. Furthermore, it appears the experiment would have been more meaningful if it had been allowed to continue longer — these plants just 2 months old, which had been started from tillers (p. 260), might not have had adequate time to recover from transplant shock.

Sincerely,

Neil E. Hoffman
Regulatory Division Director

cc: Keith Redding, Regulatory Affairs Manager
Monsanto Company

(Note: This final letter is revised slightly from the one sent on October 9, 2003, to clarify those matters relating to the petition itself.)

November 10, 2003

Neil Hoffman, Ph.D.
Regulatory Division Director
USDA/APHIS/BRS
4700 River Road, Unit 147
Riverdale, MD 20737-1236

Subject: Response to APHIS/BRS review of USDA petition to deregulate glyphosate tolerant creeping bentgrass event ASR368 (#03-104-01p)

Dear Dr. Hoffman,

On April 14, 2003, The Scotts Company and Monsanto petitioned the United States Department of Agriculture to deregulate glyphosate-tolerant creeping bentgrass event ASR368 (#03-104-01p). The data and information submitted to the agency provided evidence that other than tolerance to glyphosate, event ASR368 is not different from commercial or conventional creeping bentgrasses. On October 21, 2003, APHIS/BRS sent a letter to The Scotts Company and Monsanto stating that the submitted revised petition provided substantial data and information to show that the creeping bentgrass line genetically engineered to be resistant to glyphosate is not otherwise weedier than its parents. Furthermore, APHIS/BRS is not aware of other experiments to perform that might substantially further their understanding as to any unexpected weediness characteristics of line ASR368 relative to its parents and other creeping bentgrass cultivars. On this basis APHIS/BRS intends to deem the petition "complete".

Also in this correspondence, specific items were identified to which The Scotts Company and Monsanto were given the opportunity to respond. This document contains our response to those items.

Should you have any additional questions or concerns, please do not hesitate to contact either Terry Stone at 937-578-5447 or Keith Reding at 314-694-6615. Thank you for this opportunity to comment.

Sincerely,

Terry Stone by H.K.R.
The Scotts Company

Keith Reding, Ph.D.
Monsanto Company

RESPONSE TO COMMENTS BY APHIS/BRS

<u>P 31 line 9 and similarly p. 65 line 36</u>: Clarify use of the term "rare" to define the potential of *Agrostis stolonifera* to form hybrids with related species.

On page 31 line 9, in this overall summary statement for Section II of the petition, The Bentgrass Family, "possible" may be substituted for "rare". The sentence may now read:

> "...the formation of hybrids with related species is possible but declines precipitously with increasing distance from the pollen source and hybrid fertility is generally low."

On page 65 line 36, this sentence may be re-stated as follows:

Finally, although *A. stolonifera* can cross with related species to form hybrids, they are largely sterile, typically intermediate to the parent species and pose essentially no risk to agriculture or the environment.

<u>P. 31 line 12</u>: Clarify the statement "the species neither exhibits characteristics of a weed nor has a history of weediness".

As APHIS indicates, *A. stolonifera* may exhibit some characteristics of a weed. Although it may be considered an undesirable plant in turfseed production or in turfseed for lawn use, it is not considered a major weed in U.S. agricultural, the country in which we are seeking deregulation (Holm *et al.*, 1979; Uva *et al.,* 1997; USDA Federal Noxious Weed list, 2003).

<u>P. 56 line 33</u>: Clarify the circumstances in which *A. stolonifera* is a weed.

The section of page 56 referred to by APHIS may be restated as follows:

Agrostis stolonifera may be considered an economic weed in a limited number of circumstances: (1) as an escape from and/or volunteer in seed production fields in regions of diverse grass species production for pedigreed seed and (2) when growing or spreading into areas planted to other turfgrass types. Although the species may be a colonizer of nonagricultural habitats, its presence in these areas results from disturbances related to human activities, i.e., its use in animal-based agriculture and residential or commercial lawns. *A. stolonifera* is not considered a major weed in other nonagricultural habitats or agricultural situations.

<u>P. 58 line 27</u>: Clarify if the planting dates of the seed establishment studies were optimal

Agrostis stolonifera has a longer juvenile stage (greater time required to achieve an appropriate size for reproductive induction) and therefore requires an earlier fall planting date than most other turfgrass species for optimum economic seed production. However the intent of this study was to assess seed establishment, not economic seed production. The climatic conditions at the time these studies were initiated were suitable for the germination of creeping bentgrass seed. Therefore, the timing of the planting had no impact on the quality or interpretation of the results.

In addition, Rmax should not be used as a quantitative value but rather as a relative index, based on transformed data taken from plants grown under near ideal growth conditions. Rmax is an attempt to remove the potential bias of plant size from the discussion of ecological and evolutionary significance of the ability of a plant species to accumulate dry weight. In clarification, the results of Grime and Hunt (1975) show no significant differences between *Agrostis stoloniofera*, *Poa trivialis*, *Taraxacum officinale* and *Trifolium repens* (the upper and lower confidence intervals, see page 408 of the citation). When environmental stresses are used in Rmax generation, most species show decreased Rmax values (see Hunt *et al.*, 1987 to compare the relative stress response of *A. stolonifera* versus *A. capillaris* in the Appendix on p. 59). Therefore stress can have a major impact. Timing of stress, especially immediately after emergence can greatly affect plant survival as well as dry weight accumulation in *A. stolonifera* (Cattani, 2001).

<u>P. 58 line 39</u>: Reconcile implications made in the petition regarding *A. stolonifera's* difficulty to establish in the Willamette Valley outside of production sites in the absence of irrigation in light of literature cited by APHIS.

Agrostis stolonifera's presence as a naturalized component of the North American flora is not in dispute. However, the references cited point to a human disturbance and animal-based agriculture relationship to its occurrence. It is important to note that Hitchcock (1950) suggests *A. stolonifera* may be native to North America. Questions as to the origin of the naturalized *A. stolonifera* reported in the cited references are not easily answered. The presence of this species pre-dates the practice of seedsmanship in Oregon (see the cultivar description of 'Seaside' creeping bentgrass). There is an assumption by many that this species is entering natural areas; however, it would be more accurate to say that *A. stolonifera's* presence in an ecosystem is the result of human disturbance and use of the habitat for animal-based agricultural purposes (Frenot *et al.*, 2001). In many cases, *A. stolonifera* has provided ecological benefits, i.e., the reduction of soil erosion due to the impacts of animals utilized in agriculture and the animals altering the ecosystem, including soil nutrient content.

<u>p. 64 line 4</u>: Reconcile implications made in the petition regarding *A. stolonifera's* difficulty to vegetatively establish in light of literature cited by APHIS.

The most likely means of introduction into an ecosystem is through seed. Introduction via stolon pieces is unlikely, although vegetative spread, once established, would be the likely mode of persistence within an ecosystem (Frenot *et al.*, 2001). *A. stolonifera* can be found in habitats similar to its native areas, predominantly moist pasture type settings, and would appear to follow human agricultural disturbance (Gremmen *et al.*, 1994; Frenot *et al.*, 2001) or urban development (Mueller-Warrant et al., 2002).

Although Dore and McNeill (1980) are cited regarding the spread of bentgrasses in Canada via vegetative plant material or seeds, this reference should be viewed in light of the country's law regarding crop seeds. The Canadian Seeds Act allows for a component of "other crop" of up to 2% (No.1 Certified Seed Class) and 4% (No.2 Certified Seed Class) by weight (Canada Seeds Act; Table XII). The Act also provides for the use of *Agrostis* species in Canadian Lawn Seed Mixtures (see attached Table XIV Part II of the Canada Seeds Act). The continued use of *Agrostis* species in the class of lawn seed found most often in nurseries and in building and hardware stores (Canada No. 1 and No. 2 Lawn Grass Mixture) may help account for the presence of these species in the landscape. Similarly, as *Agrostis* species are considered "other crop", they may be found in forage grass seed (e.g. Timothy, *Phleum pratense* L.).

The introduction of *A. stolonifera* onto the sub-Antarctic islands occurred via animal-based agriculture (Frenot *et al.*, 2001). As noted by the authors, *A. stolonifera* spread out of these habitats and in some instances within these habitats. However, once it is established in a habitat, vegetative spread is the primary mode of colonization (no viable seed produced).

Jones *et al.* (2003) is a review and only reports on literature citations. The citation in question was a report for the government of Tristan da Cunha. Therefore the statements that are found in Jones *et al.* (2003) are not a scientific report of the supposed 'invasiveness' of *A. stolonifera* but rather, are a second-hand account of findings. The proper citation should be the Cooper and Jones (1994) report to the Government of Tristan da Cunha. Nonetheless, we do not question that *A. stolonifera* has been found as a naturalized species on Gough Island; however, its introduction was likely intentional via animal-based agriculture.

Mueller-Warrant *et al.* (2002) cites only *Agrostis* sp. and not *A. stolonifera*. *A. capillaris* (colonial bentgrass), *A. gigantea* (redtop), and other *Agrostis* spp. would be included in this reference. Therefore, other than the presence of *A. stolonifera* in the Willamette Valley of Oregon, this reference should not be used to quantify the occurrence of, or make inferences to, *A. stolonifera*. The letter from M. Jordan referenced in your correspondence has a similar deficiency (refers to *A. capillaris*). Nonetheless, the occurrence of these species should be examined in light of *Agrostis* species used in seed mixtures planted in the Pacific Northwest for irrigation bank stabilization (e.g., Agency Plains District in Jefferson County, Oregon). They have also been used in residential or

commercial turfgrass areas (Miltner *et al.*, 2003; Reinhold *et al.*, 2003). This usage may account for the common occurrence of these species in the more populated areas of Oregon as noted by Mueller-Warrant *et al.* (2002). Finally, despite the citations by Mueller-Warrant (2002) and M. Jordan and their intentional use, DiTomaso and Healy (2003) list none of the commonly produced *Agrostis* species as weeds in their recently published text 'Aquatic and Riparian Weeds of the West'.

P. 127 lines 28 and 32: Clarify what the first progeny population is as the R1 and F1 are defined as the second progeny populations.

The ASR368 R0 plant (primary transformant) and all vegetative clones of this event originally regenerated from tissue culture, represent the first generation of event ASR368. Subsequent sexual generations are numbered relative to the R0 (first) generation.

P. 127 line 36: F2. Clarify whether the pollen from F1 ASR368 was derived from a single plant or a population of plants.

The F2 represents the third generation progeny population of ASR368 plants resulting from the hybridization of conventional Elite Parent Plants with a population of F1 ASR368 progeny.

Pp. 136-138 Tables VI.A.3-5 and pp. 138-139 Tables VI.A.6-8: Clarify the term "survivability" and why more optimal conditions were not used for the assays (pp. 136-138) or why the tests were conducted so late in the season (pp. 138-139).

The term "survivability" in the context of this petition section and in Tables VI.A.3-5 is used as a relative measure of the plant establishment from seed versus the potential for establishment from the pure live seed count planted. Pure live seed counts are based on standard germination tests of the seed lots in the trial multiplied by the number of seed units planted in each plot. Survivability is calculated based on the number of plants actually found within each plot on any given date. In order to avoid undue disruption of plots, no attempts were made to track individual plants to determine if a seed that germinated and was counted on one date actually survived until the next observation date. Consequently zero survivability was recorded on some dates.

Seed planting dates used in Oregon during 2000 and 2001 coincide with the approximate start of the rainy winter season in the Pacific Northwest. The objective of the experiments was to optimize germination and establishment under natural conditions so earlier seeding dates were not used. Earlier seeding dates would coincide with dry weather patterns typical of September (ET_{50} moisture deficit) that are unlikely to result in optimum seedling germination or establishment (see tables VI.B.6 vs. VI.B.10). If seed were planted earlier and an unlikely and infrequent precipitation event occurred, it is likely that germination would initiate, but would result in higher seedling mortality since consistent moisture is not expected until October or November. Bentgrass seed growers in the Willamette Valley usually provide supplementary irrigation to expedite germination and maintain rapid establishment of new seed production fields.

Bentgrass seedlings can establish and survive under optimum environmental conditions. However, bentgrass is unlikely to germinate and establish under unmanaged conditions (Jonsdottir, 1991; Howe and Snaydon, 1986) and does have difficulty even under highly managed systems (Kendrick and Danneberger, 2002). For example, a comparison of seedling establishment under irrigated versus non-irrigated conditions initiated during September of 2001 in Oregon (pages 141 – 146 of the petition) provides evidence of the importance of irrigation in the Willamette Valley for improved fall seedling establishment. Germination and establishment under optimized environmental (temperature and moisture) conditions is observed in trials reported in section VI.G. (pages 222 – 231 of the petition). Field studies provide additional perspective on whether germination and establishment is more or less likely to occur than traditional species or at what frequency germination and establishment of any bentgrass is likely to occur, if at all, in more naturalized conditions.

<u>P. 142 line 6</u>: Clarify why the overall seed survivability of the Spring 2002 irrigated and non-irrigated plantings is so low even in the irrigated bare-soil plots.

Supplementary irrigation was only provided during the initial eight-week establishment period when up to 1" of water was provided from a combination of natural precipitation and supplementary irrigation (except for a soaking irrigation event near the end of the study to revive plants for final counts). The objective of the early maintenance was to provide a more optimized condition for bentgrass emergence in spring before the typical dry summer season started. Germination and establishment of bentgrass is relatively slow compared to other grass species and bentgrass seedlings are inconspicuous and may not be distinguishable from other grassy weeds until about 8-10 weeks after emergence. The natural conditions of the Willamette Valley during summer were not conducive to further germination and survival of bentgrass seed.

The main conclusion from the studies is that ASR368 is similar to conventional commercially available cultivars. However, the results provide further evidence that creeping bentgrass requires more optimum moisture conditions than what were provided artificially in order to establish and persist in the Willamette Valley. Bentgrass can be established in the Willamette Valley on golf courses and seed fields; however, it is unlikely to be very successful without adequate moisture for an extended timeframe. Golf courses may irrigate new greens five or more times each day in order to optimize germination and establishment. Seed testing provides for optimum moisture and temperature conditions in order to determine maximum germination potential of a seed lot. Natural conditions in unmanaged ecosystems infrequently provide optimum conditions for the extended timeframe necessary for *Agrostis stolonifera* establishment and persistence.

The following references provide further discussion of the competitiveness of creeping bentgrass seedlings: Kendrick and Danneberger (2002), Cattani and Struik (2001), Howe and Snaydon (1986), Jonsdottir (1991), and Bullock et al. (1994).

<u>Pp. 150-151 Tables VI.B.1-2</u>: Clarify the methodology employed in Experiments I and II.

Experiment II was not a "follow-up experiment" to Experiment I as inferred by APHIS. Experiments I and II (Tables VI.B.1 and VI.B.2) in Section VI.B.1 were initiated as concurrent experiments. Repetitions of Experiment I were initiated on December 12, 19 and 28, 2001 and repetitions of Experiment II were initiated on December 11, 2001, January 3 and February 7, 2002. As these experiments were run independently of each other, Experiment II was not influenced in any way by the results of Experiment I.

ASR368 establishment was not significantly different from the commercial cultivars Penn A-4, Penncross and SR1020 in Experiment I and is, therefore, unlikely to demonstrate any additional competitive ability compared to commercial cultivars. The results of Experiment I were confirmed in Experiment II during 2001 – 2002, although with different comparators representative of the non-transformed organism, *Agrostis stolonifera.*

The vegetative establishment experiments conducted during 2002 - 2003 in eight different environments (four irrigated and four non-irrigated) (Tables VI.B.3, 4, 5, 6, 7, 8, 9 and 10) further confirm that the potential for vegetative establishment of plants derived from ASR368 (two different generations of progeny) is not significantly different from that of several accepted commercial cultivars and a population consisting of EPPs used in crosses with ASR368 to generate F1 and F2 generation progeny.

Furthermore, the 2002 - 2003 experiments provide additional evidence that an extended optimum moisture regime is critical for the vegetative establishment and persistence of *Agrostis stolonifera.*

<u>P. 149</u>: Clarify the background of the ASR368-A and ASR368-B populations used as the source of stolon nodes in Experiment II (Table VI.B.2).

ASR368-A and ASR368-B stolons were randomly harvested from 50 progeny plant populations (50 different genotypes) each derived from seed harvested from a topcross of ASR368 F1 progeny to two different maternal Elite Parent Plants, EPPs (see Fig V.14 on page 115 for description of EPP). The two EPP plants are believed to trace from two different commercial cultivars. EPPs V15-2-5 and V4-1-6 are the conventional maternal parent plants for populations A and B, respectively. EPP 15-2-5 traces to a single plant segregant from cv. Pennlinks. EPP V4-1-6 traces to a single plant segregant from cv. Penneagle. EPP plants are likely to be highly heterozygous plants that segregate for multiple characteristics when crossed to other bentgrass genotypes. EPP lines were selected for crosses with ASR368 based upon good turf performance in progeny turf evaluations during 1998 - 2000.

EPP V15-2-5 and V4-1-6 progeny (F2 ASR368) were harvested after being pollinated by a population of F1 ASR368 progeny (heterogeneous and heterozygous population

hemizygous for the *cp4 epsps* gene derived from crosses of ASR368 R0 with four EPPs). RR progeny used as a source of stolon nodes were initially harvested as seed from the EPP plants and then selected for Roundup herbicide tolerance by spraying the segregating seedling populations twice with Roundup herbicide to identify and confirm plants derived from event ASR368 (RR).

<u>Pp. 153-157 Tables VI.B.3-10</u>: Clarify the format of the data presented in these two tables.

Tables VI.B.3 through VI.B.10 all present data in the same format. The variation in the number of nodes establishing among sites is due to the differences in actual establishment as impacted by the use of irrigation to supplement natural precipitation versus trial locations with only natural precipitation and no supplemental irrigation.

<u>P. 157 VI.B.3 Second paragraph, second sentence</u>: Clarify the background of the RS population used in this experiment.

The RS population designation was used in the petition to refer to both segregants and to other conventional plants used in experiments. The RS is described on page 127 of the petition as:

> "A glyphosate susceptible plant identified among the segregating ASR368 F1 or R1 progeny population. These "RS" plants or "null segregants" did not inherit the *cp4 epsps* gene. RS has also been used to refer to other non-transgenic conventional plants used as comparators in experiments."

For clarity, the sentence on page 157, section VI.B.3, second paragraph and second sentence should read:

> "Significant differences between ASR368 RR and both RS segregants and cv. Crenshaw were detected for vegetative establishment in Experiment I. However, ASR368 RR was not significantly different from the three other commercial cultivars (Penn A-4, Penncross and SR-1020) and Crenshaw was not significantly different from Penncross, SR-1020 and ASR368 RS."

Although no RS segregant progeny were included in Experiment II due to lack of availability of similar age plants maintained in similar fashion to the ASR368 A, ASR368 B, Penneagle and Backspin populations, commercial cultivars are considered to be RS phenotypes. The heterozygous nature of *Agrostis stolonifera* provides for a range of phenotypes that must be considered as representative of the non-transformed organism, *Agrostis stolonifera*, when determining relative performance.

<u>Pp. 171-173 Tables VI.c.13-16</u>: Clarify whether the data are for RR, RS or a mixture of both, and whether independent data were available for both RR and RS.

The ASR368 R0 is derived from vegetative clones of the glyphosate-tolerant primary transformant ASR368 and consequently, RR. The ASR368 F1 progeny population are glyphosate-tolerant plants selected for tolerance to Roundup and consequently, also RR. All other treatments in both experiments are conventional, Roundup herbicide-sensitive plants representing the non-transformed organism (*Agrostis stolonifera*) and non-transformed related *Agrostis* species.

<u>P. 256 line 35</u>: Include a more recent reference to support the statement the Willamette Valley has been the predominant creeping bentgrass seed production area of the U.S. for the past 75 years.

Oregon Department of Agriculture. 2001. Oregon Agricultural Statistics 1999-2000. Oregon Agricultural Statistics Service. Salem, OR.

<u>P. 260 line 8</u>: Clarify whether these are 20 clones or 20 genetically distinct seedlings.

Three replications, each containing 20 genetically identical plants of each seed lot were arranged in a randomized complete block design.

<u>Pp. 263-264 Tables VII.C.1a-b and C.2</u>: Clarify that the data on percentage of RR plants and percentage of hybrids would need to be multiplied by 2 to reflect that only 50% of the pollen from the RR plants carry the glyphosate tolerance gene.

The RR plants are hemizygous and therefore only 50% of the pollen carries the glyphosate-tolerant gene.

<u>P. 267 Table VII.C.6:</u>

The transformation of the diameter data to % ground cover created an anomaly for % cover since plants within rows were planted closer than the distance between rows. Plant diameter was measured across (between) the rows instead of along the rows where plant growth overlapped and precluded any measurement in that direction.

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Additional Information Provided to USDA

- Summary of Alternative Pesticides for Removal of Roundup Ready Creeping Bentgrass in Undesired Areas on Golf Courses, Grass Seed Production, and other Crop and Non-crop Areas
- Pest Risk Assessment for Agrostis stolonifera L. Creeping Bentgrass, genetically modified to include glyphosate resistance by Dr. Alan Tasker, PPQ, APHIS, USDA
- Expert Letter from Dr. Zac Reicher, Associate Professor, Turfgrass Extension Specialist, Purdue University
- Expert Letter from Dr. Stephen Hart, Assistant Extension Specialist, Rutgers University
- Expert Letter from Dr. Suleiman Bughara, Assistant Professor, Turf Grass Geneticist and Breeder, Michigan State University
- Poster Abstract by Carson et al. entitled “Selective Control of Creeping Bentgrass in True Putt Creeping Bluegrass”, presented at the 2003 American Society of Agronomy Annual Meeting

August 13, 2003

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Subject: Summary of Alternative Pesticides for Removal of Roundup Ready Creeping Bentgrass in Undesired Areas on Golf Courses, Grass Seed Production, and other Crop and Non-crop Areas

On April 14, 2003, Monsanto and The Scotts Company petitioned the United States Department of Agriculture to deregulate glyphosate tolerant creeping bentgrass event ASR368 (USDA petition #03-104-01p). The data and information submitted to the agency provided evidence that other than tolerance to glyphosate, event ASR368 is not different from commercial or non-transgenic creeping bentgrasses. This document summarizes the chemical pesticides registered by the Environmental Protection Agency (EPA) for the control of perennial grasses, including bentgrass species, in golf courses, grass seed production, other crop (food or feed) and non-crop areas.

Potential Weediness of *Agrostis stolonifera*

In general, *A. stolonifera* is not considered a serious, principal or common weed in the continental U.S. (Holm *et al*., 1979) and none of the *Agrostis* species appears on the USDA Federal Noxious Weed list (USDA, 2002).

In grass seed production, all volunteer grasses, including *A. stolonifera*, may be considered weeds when they occur in production fields of other grass types and are undesirable due to the negative impact on crop value in light of certification standards. However, *A. stolonifera* is considered an uncommon weed in other turfgrasses for several reasons: (1) it develops at a slower rate than other grasses, (2) is the last grass to mature in the Willamette Valley of Oregon, where more than 95% of the production of all creeping bentgrass occurs and (3) has an extremely small size in comparison to other commonly grown turfgrasses produced for seed. Most *A. stolonifera* seed that might contaminate seed of these other species is removed routinely in combines and various seed cleaners in downstream conditioning operations.

Agrostis stolonifera has never been considered a serious weed of other turfgrasses; however, its presence may be undesirable, causing non-uniform patches that deteriorate in quality and aesthetics when mowed above one-half inch (Watschke, 1995). The most common scenario is lateral growth of *A. stolonifera* off of golf putting greens into surrounding turfgrass. Nonetheless, *A. stolonifera* rarely earns the attention of professional turfgrass managers to the extent of crabgrass or dandelion because it is a relatively uncommon and localized concern.

In agricultural crops, tillage operations, herbicide programs and economical water management in most systems prohibit establishment and spread of *A. stolonifera*.

Chemical Pesticides Registered for the Control of *A. stolonifera*

There are a number of chemical herbicides and fumigants registered for the control of perennial grasses, including bentgrass species, in golf courses, grass seed production, other crop (food or feed) and non-crop areas such as roadsides, utilities right of way, around farm buildings, equipment, etc. (Table 1). However, since bentgrasses are considered minor weeds in agriculture, other than in grass seed production, many pesticides do not specifically include them on the label. Nonetheless, FIFRA Section 2(ee) was written to permit the use of a pesticide in an approved crop to be used for the control of pests not specifically identified on the label.

Table 1 lists a number of herbicides and fumigants that can be applied to control creeping bentgrass or event ASR368 in grass seed production, golf courses, other food or feed crops and non-crop areas if desired. Direx and GramoxoneMAX, are broad-spectrum herbicides labeled for pre-plant and/or pre-emergent application in grass seed production, and Direx, Rely, Goal and Sinbar, are labeled for post-emergence use. On golf courses Envoy, Finale and Fusilade II are registered for perennial grass control as well as the fumigants, Vapam, Methyl Bromide (Terr-O-Gas) and Basamid. Finally, Poast, Assure, Fusilade DX, Envoy and several other pesticides are currently registered for application in other crop and non-crop areas.

In addition to these currently registered pesticides, Monsanto and The Scotts Company are working with other chemical pesticide manufacturers and university scientists to develop recommendations for grass seed growers and golf course managers desiring to eliminate conventional creeping bentgrass or creeping bentgrass tolerant to glyphosate. Therefore, considering the minor weed status of creeping bentgrass and the chemical and mechanical options currently available, its removal from areas in which it is undesired is not a concern today and will continue to be manageable upon the deregulation and commercialization of event ASR368.

Should you have any questions with regard to this information, please do not hesitate to call me at XXX-XXX-XXX.

Sincerely,

Terry Stone
Director, Biotechnology Regulatory Affairs
The Scotts Company

Keith Reding, Ph.D.
Regulatory Affairs Manager
Monsanto

Table 1. Chemical pesticides registered for the control of perennial grasses on golf courses, grass seed production, other crop (food or feed) and non-crop areas.

Product	Reg. No.	ai	Product specifically labeled for use in/on:			
			Golf courses	Grass seed production	Other crop	Non-crop
Envoy (Valent)	59639-78	Clethodim	Yes	No	No	Yes
Envoy is labeled for annual and perennial grass control in a number of non-food crop areas, including non-crop or non-planted areas around golf courses and ornamental nurseries.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Finale (Bayer)	432-1229	Glufosinate	Yes	No	No	Yes
Finale is labeled for non-selective control of annual and perennial grass and broadleaf weeds in non-crop areas such as golf courses and a number of non-food crop areas including ornamental nurseries.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Fusilade II (Syngenta)	10182-393	Fluazifop	Yes	No	No	Yes
Fusilade II is labeled for annual and perennial grass control in turf areas of golf courses and non-food crop areas of turfgrass sod farms and ornamental nurseries.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Direx (Griffin)	1812-257	Diuron	No	Yes	Yes	Yes
Direx is a broad spectrum herbicide labeled for annual and perennial grasses and broadleaf weeds in a number of food and perennial and some annual grass seed crops, which include pre-and post-emergent use on bentgrasses, Kentucky bluegrass, annual and perennial ryegrass and fescues. Direx is also labeled for use on a number of non-crop areas including roadsides and utility rights-of-way.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Goal 2 XL (Dow)	62719-424	Oxyfluorfen	No	Yes (SLN)	Yes	Yes
Goal 2XL is labeled for weed control in fallow beds prior to planting a number of food crop and in non-crop areas such as along fence rows, on farmsteads and in utility rights-of-way. An SLN 24C label is approved in the state of Oregon for Use in Certain Established Perennial Grasses Grown for Seed. This SLN label is valid until December 31, 2006. It allows the use of Goal 2XL for perennial grass seedling control in established perennial grasses grown for seed production. More specifically, this label is for control of seedling stage perennial grasses within established perennial grasses grown for seed.						

Table 1. Continued.

Product	Reg. No.	ai	Product specifically labeled for use in/on:			
			Golf courses	Turf seed production	Other crop	Non-crop
GramoxoneMAX (Syngenta)	100-1074	Paraquat	No	Yes	Yes	Yes
GramoxoneMAX is labeled for annual and perennial broadleaf and grass weed control for applications prior to planting or prior to emergence of a number of food crops, including on fallow land. Uses also include preplant (only) applications for seedbed preparation in grasses grown for seed (grazing or feeding of treated material is prohibited), and suppression of sod and undesirable grasses prior to pasture reseeding.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Turf seed production**	**Other crop**	**Non-crop**
Rely (Bayer)	264-652	Glufosinate	No	Yes	No	No
Rely is labeled for broadleaf and grass weed control in apples, grapes and tree nuts, and for potato vine desiccation. An SLN 24C label for Rely is approved in the state of Oregon, specifically for weed control in grass grown for seed production only. This SLN allows for broadcast applications at the 4 to 6 tiller stage in the spring, and hooded or shielded sprayers in row-middles for control of grassy weeds such as Poa spp., Manna grass and Bromus spp. The SLN expires December 31, 2003, however renewal is expected until December 31, 2007.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Select (Valent)	59639-3	Clethodim	No	No	Yes	Yes
Select is labeled for annual and perennial grass weed control in a number food crops, in agricultural fallow land, and non-crop or non-planted areas such as rights-of-way.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Poast (BASF)	7969-58	sethoxydim	No	No	Yes	Yes
Poast is labeled for annual and perennial grass weed control in a number of food crops, on fallow land and on a number of non-crop areas including roadsides and utility rights-of-way.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Sinbar (DuPont)	352-317	Terbacil	No	Yes	Yes	No
Sinbar is a broad spectrum herbicide labeled for annual and perennial grasses and broadleaf weeds in a number of food and forage crops. A supplemental label for use on grass seed crops in WA, ID and OR has also been established.						

Table 1. Continued.

Product	Reg. No.	ai	Product specifically labeled for use in/on:			
			Golf courses	Grass seed production	Other crop	Non-crop
Eptam (Syngenta)	10182-220	S-ethyl, dipropyl-thiocarbamate	No	No	Yes	No
Eptam is a broad spectrum herbicide labeled for broadleaf weeds, annual grasses, bermudagrass and quackgrass on a number of food and forage crops. Perennial weeds must be chopped up and turned over prior to treatment to obtain adequate control.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Assure II (Dupont)	352-541	Quizalofop-p-ethyl	No	No	Yes	Yes
Assure II herbicide is currently labeled for annual and perennial grass control in a number of food crops and in non-crop areas such as fencerows, roadsides, equipment storage areas and similar areas. Grazing or feeding of treated forage, hay and straw is prohibited. A state-specific (includes OR) supplemental label is approved for grass weed control in certain non food/non feed crops grown under contract for seed production only; the listed crops are: alfalfa, onion, carrot, garlic, Swiss chard, spinach, radish, Chinese cabbage, and red beets.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Fusilade DX (Syngenta)	100-1070	Fluazifop	No	No	Yes	Yes
Fusilade DX is labeled for annual and perennial grass control in a number food crops, in agricultural fallow land, and on agricultural non-crop areas, such as around farm buildings and equipment.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Grass seed production**	**Other crop**	**Non-crop**
Arsenal (BASF)	241-299	Imazapyr	No	No	No	Yes
Arsenal is currently labeled for annual and perennial grass and broad leaf weed and brush control in site preparation, conifer release and along roadsides in forestry operations; no other use sites are included.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Turf seed production**	**Other crop**	**Non-crop**
Outrider (Monsanto)	524-500	Sulfosulfuron	No	No	No	Yes
Outrider is labeled for annual and perennial grass and broadleaf weed control in a number of non-crop sites, including roadsides, utility rights-of-way and similar sites.						

Table 1. Continued.

Product	Reg. No.	ai	Product specifically labeled for use in/on:			
			Golf courses	Turf seed production	Other crop	Non-crop
Vapam HL (AMVAC)	5491-468	Metam sodium	No	Yes	Yes	No
Vapam is a general soil fumigant labeled for use on all crops to suppress or control soil-borne diseases, nematodes and weeds.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Turf seed production**	**Other crop**	**Non-crop**
Terr-O-Gas (Great Lakes Chemical Corp.)	5785-25	Methyl bromide	Yes	Yes	Yes	No
There are a number of manufacturers of methyl bromide. The pesticide is employed as a general soil fumigant for crops to suppress or control soil-borne diseases, nematodes and weeds on food and non-food crops and turfgrass. Terr-O-Gas and other methyl bromide formulations are restricted use pesticides due to their acute toxicity.						
Product	**Reg. No.**	**ai**	**Product specifically labeled for use in/on:**			
			Golf courses	**Turf seed production**	**Other crop**	**Non-crop**
Basamid (BASF)	7969-99-51036	Tetrahydro-3,5,-dimethyl-2H-1,3,5-thiadiazine-2-thione	Yes	No	Yes	No
Basamid is a soil fumigant for establishing or renovating turf sites including golf course greens and tees, ornamental sites and field nurseries eg. forest, non-bearing and ornamental trees, Christmas tree seedlings, shrubs, bedding plants and non-bearing crops to suppress or control soil-borne diseases, nematodes and weeds.						

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Pest Risk Assessment for

Agrostis stolonifera L.

Creeping bentgrass, Genetically modified to include glyphosate resistance

Reviewers:
Erich Rudyj, USDA, APHIS, PPQ
Polly Lehtonen, USDA, APHIS, PPQ

Adapted into risk assessment format by:

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Animal and Plant Health Inspection Service
Plant Protection and Quarantine
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Pest Risk Assessment for *Agrostis stolonifera L.* Genetically modified to include glyphosate resistance

This risk assessment conforms to the USDA, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (APHIS, PPQ) format for weed risk assessment. APHIS uses risk assessments as a basis for weed exclusion decisions; determining whether or not a weed species should be listed in (or de-listed from) the noxious weed regulations. Plant Protection and Quarantine (PPQ) risk assessment procedures are harmonized with those of the North American Plant Protection Organization (NAPPO) and the International Plant Protection Convention (IPPC) of the United Nations Food and Agriculture Organization (FAO). Our use of biological and phytosanitary terms (*e.g.*, introduction, quarantine pest) conforms with the *NAPPO Compendium of Phytosanitary Terms* (NAPPO 1995) and the *Definitions and Abbreviations* (Introduction Section) in *International Standards for Phytosanitary Measures, Section 1—Import Regulations: Guidelines for Pest Risk Analysis* (FAO 1995).

Stage 1: Initiating Pest Risk Assessment (PRA) Process

Step 1. Document the Initiating Event(s) for the PRA.

This risk assessment was developed in response to a petition from International Center for Technology Assessment, submitted by Andrew Kimbrell, Executive Director, Joseph Mendelson, III, Legal Director, and Peter T. Jenkins, Attorney Policy Analyst. Information from the petition was adapted to this format and included with additional information. The PRA area is the United States.

Step 2. Identify and Cite Previous Risk Assessments.

This is the first USDA pest risk assessment for this species related to Federal Noxious Weed regulatory status.

Step 3. Establish Identity of Weed.

Scientific Name: Order, Family, Genus, and species:

Cyperales, Poaceae, *Agrostis stolonifera L.*
Genetically modified to include glyphosate
resistance

Synonym(s):
Agrostis alba L. var. *palustris* (Huds.) Pers.; *Agrostis alba* L. var. *stolonifera* (L.) Sm.; *Agrostis maritima* Lam.; *Agrostis palustris* Huds.; *Agrostis stolonifera* L. var. *compacta* Hartman; *Agrostis stolonifera* L. var. *palustris* (Huds.) Farw.

Common name(s):
Creeping bentgrass genetically modified to include glyphosate resistance.

Description, general morphology:
A prostrate and low-growing, glabrous perennial with numerous long stolons spreading along the surface of the ground, branching and rooting at the nodes; forming mats of foliage in moist situations in pastures and ditches, generally on sandy soils.

Leaves rolled in the bud-shoot. **Sheath** not compressed, not keeled, glabrous, smooth, pale green or purplish, shorter than or equaling the internode in length on the vegetative shoots, split with hyaline margins. **Auricles** absent. **Collar** distinct, glabrous, pale green, usually oblique. **Ligule** membranous, thin, 1.5 to 3 mm. long, rounded or obtuse, finely lacerate-toothed or entire, minutely hairy on the back. **Blade** 1.5 to 4 mm. wide, 3 to 10 cm. long, erect, flat, tapering, distinctly ridged on upper surface, slightly keeled on lower surface, scabrous on the surfaces and margins.
[http://www.caf.wvu.edu/~forage/library/cangrass/page56.htm]

Genetic modification of creeping bentgrass to include glyphosate resistance would not be expected to change these traits.

Pertinent information regarding life history, including growth, development, means of reproduction and dispersal:

Creeping bentgrass is a fast-growing perennial species which is biologically and ecologically variable, adaptable, and robust, with vegetative spread and reproduction by stolons (horizontal above-ground stems or runners), wind-pollinated flowers, and tiny seeds dispersed by wind, water, and animals [Sell & Murrell 1996]. Genetic modification of creeping bentgrass to include glyphosate resistance would not be expected to change these traits.

Preferred habitat and climatic tolerance:

Creeping bentgrass is a cool season grass used for high quality putting greens, fairways, bowling clubs, and for closely cut lawns [Wipff and Fricker, 2001]. Genetic modification of creeping bentgrass to include glyphosate resistance would not be expected to change this.

Native distribution:

Creeping bentgrass is native to Eurasia and North Africa [Welsh, et al. 1987]. Some authorities list the species as native to certain localities in northern north America (Canada and United States) [Harvey, 1999l Dore & McNeal, 1980; Voss, 1972; Hitchcock & Chase, 1951].

Current world distribution beyond native distribution:

Creeping bentgrass is widely used on golf courses throughout the world [Young et al. 1997]. It was probably introduced to North America prior to 1750, and has become naturalized throughout the southern Canadian provinces and is listed as currently present throughout the United States [Welsh, et al. 1987; Lackschewitz. 1991; **http://www.fs.fed.us/database/feis/plants/graminoid/agrsto/all.html**]. [USDA, NRCS. 2002. The PLANTS Database].

Stage 2: Assessing pest risk

Step 4. Verify Quarantine Pest Status: Geographic Criterion.

The U.S.D.A. regulates Federal noxious weeds under authority of the Plant Protection Act (7 U.S.C. 7701 et seq.). The noxious weed regulations (7 CFR 360) list the regulated species. The Plant Protection Act (PPA) defines a regulated species as "any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment." However the PPA legislation (7 U.S.C. 7714) uses in addition the phrase "new to or not known to be widely prevalent" regarding APHIS authority to "hold, seize, quarantine, treat, apply other remedial measures to, destroy, or otherwise dispose of..." a regulated plant or article which is moving into or through the United States or interstate; or has not met post-entry quarantine requirements; or is progeny of an organism illegally moved. PPA (7 U.S.C. 7714 (f)(1)) further authorizes "REGULATIONS.—In the case of noxious weeds, the Secretary may publish, by regulation, a list of noxious weeds that are prohibited or restricted from entering the United States or that are subject to restrictions on interstate movement within the United States."

APHIS noxious weed program policy is consistent with U.S. obligations under international trade agreements. Signatory countries are obligated to base their phytosanitary measures on international standards. A glossary of phytosanitary terms and a standard for pest risk analysis have been developed by the International Plant Protection Convention (IPPC) and the North American Plant Protection Organization (NAPPO), which is a regional plant protection organization made up of the United States, Canada, and Mexico. Under IPPC, an international treaty of which the US is a signatory, a country can prohibit or restrict importation only of regulated pests, which, in the case of federal noxious weeds, are quarantine pests. A quarantine pest is defined as "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled." In the case of our noxious weeds program, APHIS lists in the regulation Federal noxious weeds which meet the definition of a quarantine pest.

Because of its wide distribution, creeping bentgrass as a species clearly does not meet this definition. The difference from other creeping bentgrass indicated in the petition by ICTA for the varieties in question is the glyphosate resistance trait. Other types of

bentgrass herbicide resistance are noted to occur in the species naturally. From a list of 26 common turf postemergence applied herbicides or herbicide combinations, non-bioengineered creeping bentgrass is noted to be partially or fully resistant to 15. This includes resistance to the postemergence applied graminicide fenoxaprop, which is noted to remove bermudagrass, centipede, and St. Augustine from creeping bentgrass [Murphy and McCarty. 1999]. Thus, resistance to various herbicides (including grass control herbicides) is common in this species, not a novel trait.

Genetic engineering allows the precise inclusion of specific traits into plant varieties. The addition of glyphosate resistance by genetic modification does not change other creeping bentgrass traits, thus the altered variety differs only in that trait from the rest of the widespread species, which has been in trade and general use throughout the U.S. (and the world) for many years. [USDA, NRCS. 2002. The PLANTS Database]. In the case of the ICTA petition, the fact that the resistance trait is inserted by biotechnology methods does not appear to have any relevance to the invasiveness of the species.

Therefore, the assessed variety does not meet the international definition of a quarantine pest ("new to or not known to be widely prevalent"). The assessment terminates at this point.

Summary and Conclusion:

Creeping bentgrass, which is common to the U.S., does not meet the international definition of a quarantine pest ("new to or not known to be widely prevalent"). Creeping bentgrass varieties genetically modified to include glyphosate resistance are not determined to differ sufficiently from other creeping bentgrass as to meet that definition. Because herbicide resistance alone would not markedly change the risk ranking or the species identity, these varieties are not assessed as appropriate for listing as Federal Noxious Weeds.

Step 8. References.

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PURDUE
UNIVERSITY

DEPARTMENT OF AGRONOMY
Crop Soil and Environmental Sciences

September 9, 2003

Domingo Riego, Ph.D.
Monsanto
1307 Cottonwood Ct
Carmel IN 46032
Via email

Dr. Riego:

This note is in response to potential concern over remediation of Roundup[tm] resistant creeping bentgrass (RRCB) contamination of non-target areas. There is potential for RRCB to spread to non-glyphosate-resistant turf stands. Traditional creeping bentgrass contamination does occur on lawns next to golf courses and in Kentucky bluegrass roughs on golf courses, normally due to poor sanitation during seeding. Currently, three applications of glyphosate is the best control method for escaped bentgrass. Our research has focused on alternatives for glyphosate in controlling bentgrass. We found at least three herbicides that provide equal control to that of glyphosate including fluazifop (Fusilade), clethodim (Select), and sethoxydim (Poast Plus) (see the attached document). I believe more research on atrazine will reveal ways to improve its effectiveness on creeping bentgrass. Furthermore, we are working on two experimental herbicides that provide some selective control of creeping bentgrass. A research project we just initiated this fall will likely perfect a strategy for selective control of creeping bentgrass with sulfosulfuron. Furthermore, since creeping bentgrass forms an easily visibly distinct patch, spot-treating with herbicides will be effective and there will be no need for broadcast applications. Therefore, even though RRCB contamination is a concern, remediation can be achieved through any of at least 3 currently labeled herbicides.

I believe RRCB is a tremendous advance for turf management that will lead to reduced management inputs, decreased water use, lower environmental risks from off-target movement of pesticides, and slashed management costs. Please contact me at xxxxxxxxxxxxxxxxxxxxxxxxxxxxx if I can answer questions or provide further information.

Sincerely,

Zac Reicher
Associate Professor/
Turfgrass Extension Specialist

School of Agriculture

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RRCB Control Alternatives Screening Trial with Current Standards and Additional Candidates

Zac Reicher and Dan Weisenberger
Purdue University
June 2003

Background/Objective

It is important to demonstrate that there are herbicidal control alternatives to Roundup for the control of Roundup Ready Creeping Bentgrass. We conducted small-plot trials to confirm non-selective control of bentgrass using current standard products compared to additional new candidates.

Site Information

Location:		William H. Daniel Research and Diagnostic Center, W. Lafayette, IN.
Soil Type:		Starks-Fincastle silt loam
Soil pH:		7.2
Soil Organic Matter (%):		
Turfgrass Species:		
Turf Condition:		
Turf Management:	**Mowing Height cm (in):**	1.25 (0.5)
	Fertilization:	
	Irrigation:	To prevent moisture stress
Testing on Site Previous Year:		None
Target Pest:		Creeping bentgrass
Growth Stage:		Mature

Application Information

Application Date:	15 July	15 Aug	17 Sep
Application Time:	7:30 AM	7:00 AM	7:30 AM
Air Temperature C^{o}(F^{o}):	22.3 (72.2)	23.3 (74)	18.1 (64.6)
Relative Humidity(%):	69	84	66
Wind Speed m s^{-1} (mph):	Calm	1.8 (4)	Calm
Soil Temperature(7.6 cm depth) C^{o}(F^{o}):	21.7 (71)	21.1 (70)	16.1 (61)
Soil Moisture:	Moist	Wet	Moist
Spray Volume L ha^{-1} (gal 1000 ft^{-2}):	407 (1)		
Spray Pressure:	35psi		
Spray Nozzle:	8001		
Spray Equipment:	CO_2 backpack		
Irrigation After Application:	None		
Experimental Design:	Randomized complete block		
Replications:	3		
Plot Size m (ft):	1.5 X 1.5 (5 X 5)		

Results

Though the data was highly variable among replications, three applications of Roundup Pro, Fusilade, Select, and Poast Plus provided 0% cover of creeping bentgrass by 5 Nov. Two applications of Roundup Pro provided 9.3% cover of bentgrass, but two applications of the Fusilade, Select, and

Poast Plus provided poor control. A single application of Atrazine gave immediate burndown, but the bentgrass recuperated to 60% cover by Nov. and multiple applications may be beneficial. Assuming Roundup resistant creeping bentgrass responds to herbicides similar to non-Roundup-resistant creeping bentgrass, 3 applications of Fusilade, Select, and Poast Plus appear to be adequate alternatives to Roundup Pro for control of Roundup-resistant creeping bentgrass.

Table 1. Percent cover of living creeping bentgrass.

Treatment	Rate of application lbs ai/A	Application timing[e]	Aug 2	Aug 15	Sep 3	Sep 18	Oct 2	Oct 13	Oct 23	Nov 5
Check			96	93	70	70	73	88	90	93
Roundup Pro	1.5[a]	0	0	0	0	3	10	15	13	23
Roundup Pro	1.5[a]	0, 4	0	1	0	2	3	6	7	9
Roundup Pro	1.5[a]	0, 4, 8	0	0	0	1	0	0	0	0
Fusilade DX 2L (Fusilade II)	0.375	0	33	57	53	78	75	83	86	90
+ COC	1[b]									
Fusilade DX 2L (Fusilade II)	0.375	0, 4	30	25	0	4	20	30	30	35
+ COC	1[b]									
Fusilade DX 2L (Fusilade II)	0.375	0, 4, 8	48	30	0	2	0	0	0	0
+ COC	1[b]									
Roundup Pro	1.5[a]	0, 4	0	1	0	1	4	6	4	8
+ Fusilade DX 2L (Fusilade II)	0.375									
Select 2EC (Envoy)	0.25	0	65	82	88	85	87	91	94	96
+ COC	1[b]									
Select 2EC (Envoy)	0.25	0, 4	63	73	0	1	10	22	30	42
+ COC	1[b]									
Select 2EC (Envoy)	0.25	0, 4, 8	80	88	3	10	0	0	0	0
+ COC	1[b]									
Poast Plus 1L (Vantage 1L)	0.375	0	75	67	58	60	67	80	82	87
+ COC	1[b]									
Poast Plus 1L (Vantage 1L)	0.375	0, 4	53	57	2	6	23	31	33	38
+ COC	1[b]									
Poast Plus 1L (Vantage 1L)	0.375	0, 4, 8	37	50	2	4	1	2	0	0
+ COC	1[b]									
Scepter 70DG (Image)	0.5	0	94	93	72	72	72	78	78	83
+ NIS0.25[b]										

Table 1. (continued)

Treatment	Rate of application	Application timing[e]	Aug 2	Aug 15	Sep 3	Sep 18	Oct 2	Oct 13	Oct 23	Nov 5
	lbs ai/A									
Scepter 70DG (Image)	0.5	0	95	96	77	65	63	79	76	79
+ MSMA 6L	2.0									
+ NIS0.25[b]										
Atrazine 4L	2.0	0	0	3	9	22	33	47	53	60
+ COC	1[c]									
Lexone 75DF (Metribuzin)	0.25	0	42	65	47	52	52	63	63	66
+ MSMA 6L	2.0									
Balance 75WG	0.2	0	80	85	58	60	60	66	70	74
Balance 75WG	0.1	0, 4	85	85	37	38	47	43	72	77
TranXit 25DF	1.0[d]	0, 4	95	95	50	43	42	48	55	60
+ NIS0.25[b]										
MON 44951 75DF	2.0[d]	0, 4	95	96	88	88	83	89	88	92
+ NIS0.25[b]										
Assure II	0.0825	0	80	85	23	43	70	85	87	88
+ COC	1[b]									
Assure II	0.048	4								
+ COC	1[b]									
LSD (0.05)			14	20	38	38	35	38	38	35

[a] Rate of application was pounds acid equivalent per acre.
[b] Rate of application was percent volume per volume.
[c] Rate of application was quarts per acre.
[d] Rate of application was ounce product per acre.
[e] Application timing is weeks after initial application with the initial application being 15 July.

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Ms. Cindy Smith
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4700 Riverdale Road
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I am writing this letter in support of the petition from The Scotts Company and Monsanto Company to deregulate glyphosate resistant creeping bentgrass (Roundup-Ready bentgrass) so it can be utilized commercially by the golf course industry in the United States. I would specifically like to address the argument put forward by opponents of this technology which is that seed production and commercial use of glyphosate resistant creeping bentgrass will lead to the development and spread of weedy bentgrass species that can no longer be effectively managed, resulting in a negative economic and ecological impact.

I am an Extension Specialist at Rutgers, The State University of New Jersey, responsible for weed management in turfgrass and ornamentals for the states of New Jersey and Delaware I work with many key groups in the "Green Industry" of both states including the golf course industry. I am supportive of the use of glyphosate resistant bentgrass for golf courses due to the many advantages glyphosate resistant creeping bentgrass will provide to this industry.

As a public weed management specialist in turfgrass and ornamentals, I recognize that on golf courses, lawns, landscapes, sod farms, and seed production fields, glyphosate is commonly used for the control of bentgrass species in areas where they are undesirable. In addition, glyphosate is often used to desiccate existing bentgrass fairways and greens in order to prepare these areas for renovation. There is also slight potential for unintended transfer of the glyphosate resistance gene via pollen to creeping bentgrass and related bentgrass species. Therefore, an important part of developing an integrated management program is the identification of alternative herbicides for the control of glyphosate resistant bentgrass.

In 2001, comprehensive studies were conducted in cooperation with The Scotts Company to identify these herbicides. Herbicides in the cyclohexanedione and aryloxyphenoxy propionate families are used to control both annual and perennial grasses in a wide variety of dicot agronomic crops. Several of these herbicides such as fluazifop (Fusilade II), clethodim (Envoy), and sethoxydim (Vantage) are also labeled for broadcast or directed applications in landscape planting beds and some turfgrass species for removal of unwanted annual and perennial grass species. Cyclohexanedione and arlyoxyphenoxy

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propionate herbicides control grass species by inhibiting the enzyme acetyl-CoA carboxylase (ACCase), which is involved in fatty acid biosynthesis and is a completely different mode of action than glyphosate.

Bentgrass species included in this study were: Bentgrass species evaluated in these studies included: glyphosate resistant creeping bentgrass hybrid, 'RR 368'; glyphosate susceptible creeping bentgrass hybrid, 'RS 368'; a mixture of commercial creeping bentgrass hybrids ('Penn A-4', 'Backspin' and 'Crenshaw'); colonial bentgrass 'SR 7100'; redtop bentgrass 'Streaker'; and dryland bentgrass 'Trust'

Research locations for these studies were the Rutgers University Horticultural Research Farm II located in North Brunswick, New Jersey and at the Scotts Company Research farm located in Merion County, Oregon. These studies were conducted twice at both locations.

In summary, this research demonstrated that fluazifop (Fusilade II), clethodim (Envoy), and sethoxydim (Vantage) all had substantial herbicide activity on all bentgrass species. In addition, there was no differential response between glyphosate resistant creeping bentgrass hybrid, 'RR 368'; and glyphosate susceptible creeping bentgrass hybrid 'RS 368' to fluazifop, clethodim, and sethoxydim, demonstrating as expected, that the glyphosate resistant gene did not confer an increase in tolerance to herbicides with a different mode of action. Lastly, both fluazifop and clethodim applied at labeled rates effectively controlled all bentgrass species with equal effectiveness to glyphosate.

The identification of fluazifop and clethodim as effective alternative herbicides, integrated into an effective educational stewardship program for the practitioners of the technology (seed producers, and golf course superintendents etc.) will insure that this technology will pose no more of an economic and ecological risk than glyphosate susceptible creeping bentgrass and related bentgrass species.

I would be very happy to provide more detailed information concerning these studies and the results we observed. Please do not hesitate to contact me if I can be of further assistance in this matter.

Sincerely,

Stephen E. Hart

Stephen E. Hart, Ph.D.
Assistant Extension Specialist

MICHIGAN STATE
UNIVERSITY

August 7, 2003

Ms. Cindy Smith, Deputy Administrator
USDA, APHIS, BRS
4700 Riverdale Road
Riverdale, MD 20737-1237

Dear Ms Smith:

I would like to voice my support to the deregulation of Roundup Ready creeping bentgrass. Since August 1999, I have been an assistant professor in the Department of Crop and Soil Sciences at Michigan State University. My main responsibility at this university is to lead the turfgrass breeding, and genetic program and to contribute to the extension program, which is one of the best in the nation. My research focuses on using molecular technology genetic research in turfgrass breeding, especially useful in developing varieties that are resistant to such disease as snow mold, dollar spot, and brown patch. Prior to August 1999, I worked as a research assistant professor at the University of Missouri. In 1998, I received the first patent in plant sciences awarded to the University of Missouri for releasing a buffalograss cultivar called MoBuff. I have over 20 years experience as a senior researcher in this field and I am a member of the Turfgrass Breeders Association, which is supportive of Roundup Ready creeping bentgrass technology.

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The Roundup Ready creeping bentgrass will provide the opportunity to apply Roundup PRO® herbicide directly onto creeping bentgrass grown for seed, sod or turf production for the control of problem weeds such as annual bluegrass, rough stalk bluegrass and others without alteration of creeping bentgrass performance, growth or environmental adaptation. The potential elimination of annual bluegrass and other weeds would result in greater seed purity and less turf disease and insect pressure on golf courses, which if realized would reduce pesticide applications and the potential for exposure of farm workers, golfers, area residents, local wildlife and the environment to pesticide.

At the Turfgrass Research Center at MSU I have tested Roundup Ready creeping bentgrass, compared it with traditional bentgrasses and have found no performance differences from traditional bentgrasses other than the benefits of Roundup PRO resistance. In addition, Dr. Wang a Post doc. in turfgrass breeding at MSU made several hundred crosses between Roundup Ready creeping bentgrass and 14 other species of *Agrostis* by bagging the heads together to force crossing and repeated these crossing experiments two times. She found very low crossing frequencies and few seeds that could

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be collected. Pollen viability of the F1 hybrid plants ranged 4% to 52% compared with 94-96% traditional creeping bentgrass. These results indicate that hybridization potential between the Roundup Ready bentgrass and other species will be very low, and germination and survival of hybrid seed is even more unlikely.

We have been able to control bentgrass with herbicides labeled for lawn and landscape other than glyphosate such as clethodim, sethoxydim, glufosinate and fluazifop based herbicides.

I would like to thank the USDA for consideration of this letter written as reaffirmation of my position for the deregulation of Roundup Ready creeping bentgrass.

Sincerely,

Suleiman Bughrara, Ph.D.
Assistant Professor, Turf Grass Geneticist and Breeder
Michigan State University

SSB/dcm

Selective Control of Creeping Bentgrass in True Putt Creeping Bluegrass. (C05-carson382247-poster)

Authors:

- T.D. Carson – *Univ. of Minnesota*[1]
- B.P. Horgan – *Univ. of Minnesota*
- D.B. White – *Univ. of Minnesota*

Abstract:

Monostands of creeping bentgrass (*Agrostis stolonifera*) often become infested with annual bluegrass (*Poa annua*). Similarly, monostands of creeping bluegrass (*Poa annua* var. *reptans*) can develop creeping bentgrass infestations. The objective of this study was to screen for herbicides that selectively control creeping bentgrass with minimal injury to creeping bluegrass. Nine herbicide treatments were applied to 17.7 cm plugs maintained at 1.3 cm in the greenhouse. The experiment was repeated twice and was a randomized complete block design with three replications. The turf was rated at three days, seven days and then weekly for quality and percent living cover. Sethoxydim was the only herbicide that provided complete control of creeping bentgrass while causing no significant reduction in the quality of creeping bluegrass. Quizalofop P-ethyl and fluazifop P-butyl also provided complete control of creeping bentgrass, but both had a negative impact on the quality of the creeping bluegrass in the second repeat. Currently, fluazifop P-butyl is the only herbicide from this group that is labeled for use on turfgrass. Further investigation is needed to determine optimum application rates.

Speaker Information: Troy Carson, Univ. of Minnesota, 305 Alderman Hall 1970 Folwell Ave., St. Paul, MN 55108; Phone: xxx-xxx-xxxx; E-mail: xxxxxxxx@xxxxxxxx.xxx

Session Information: Tuesday, November 4, 2003, 8:00 AM-10:00 AM
Presentation Start: 8:00 AM Poster Board Number: 907

Keywords: creeping bluegrass; Poa annua; creeping bentgrass; selective herbicides

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