The Scotts Company and Monsanto Company Petition (15-300-01p) for Determination of Non-regulated Status of Glyphosate Resistant ASR368 Creeping Bentgrass

Monsanto petition number:
TR054-15U1

Preliminary Plant Pest Risk Assessment

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A. Introduction

The Scotts Company and Monsanto Company (hereafter referred to as Scotts/Monsanto) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) for a determination that the genetically engineered (GE) glyphosate herbicide-resistant[1] creeping bentgrass (*Agrostis stolonifera*) event ASR368 (hereafter referred to as ASR368 CBG) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 15-300-01p, and is hereafter referenced as Scotts/Monsanto 2015a. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq*.)[2]. This plant pest risk assessment was conducted to determine if ASR368 CBG is unlikely to pose a plant pest risk. Scotts and Monsanto state that they "have no intention to and will not commercialize or further propagate such plants in the future" and "will not grant a license to or otherwise allow other entities to obtain, use, or propagate such plants" (Scotts/Monsanto 2015a).

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest or is an unclassified organism and/or an organism whose classification is unknown, or if the Administrator determines that the GE organism is a plant pest or has reason to

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[1] Scotts/Monsanto has described the phenotype of ASR368 CBG as "herbicide tolerant" and historically APHIS has also referred to GE plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America WSSA. 1998. *"Herbicide Resistance" and "Herbicide Tolerance" defined. (Technology Note)*. Weed Technology 12, pp. 789. Retrieved from http://www.jstor.org/stable/3989101 Last accessed 04/09/2013. definition of "herbicide resistance" since ASR368 CBG has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type". By the ibid. definition, "resistance (to an herbicide) may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant resistant; it is naturally resistant."

[2] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. US-FDA. 2006. *Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use*. U.S. Food and Drug Administration. Retrieved from http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096156.htm A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

believe it is a plant pest[3]. ASR368 CBG was produced via biolistic technology using a linear DNA segment from plasmid PV-ASGT08 containing two *cp4 epsps* gene expression cassettes (Scotts/Monsanto 2015a). Portions of the introduced genetic sequences come from plant pest organisms listed in 7 CFR 340.2 (i.e. the coding sequence of the 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium sp.* strain CP4, the 3' untranslated region of the nopaline synthase (NOS) gene from *Agrobacterium tumifaciens*, the promoter (35S) from Cauliflower mosaic virus (CaMV), and the coding sequence of the neomycin phosphotransferase type II gene from *Escherichia coli*) (Table V-1, p. 84-85, Scotts/Monsanto 2015a). Therefore, ASR368 CBG is considered a regulated article under APHIS regulations at 7 CFR part 340. Scotts/Monsanto conducted introductions of ASR368 CBG as a regulated article under APHIS-authorized notifications between 1999 and 2003 (Table VIII-112, p. 238-242, Scotts/Monsanto 2015a), in part to gather information to support that ASR368 CBG is unlikely to pose a plant pest risk.

Potential impacts addressed in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with ASR368 CBG and its use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if ASR368 CBG is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about ASR368 CBG related to: plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on non-target organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology'(51 FR 23302 1986; 57 FR 22984 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (US-FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on their characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (EPA-FIFRA-SAP) (7 U.S.C. 136 *et seq)* the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and

[3] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2(b).

Cosmetic Act (FFDCA) (21 U.S. Code 346). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR 156 (Labeling requirements for pesticides and devices). Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 158 - Data requirements for pesticides, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs) and part 172 - Experimental Use Permits. EPA granted an exemption from food and feed tolerance for CP4 Enolpyruvylshikimate-3-phosphate (CP4 EPSPS) synthase enzyme on April 25, 2007 (72 FR 20431). Since Scotts/Monsanto does not intend to commercialize ASR368 CBG, neither Scotts nor Monsanto are seeking, or will seek in the future, a label amendment from EPA with respect to ASR368" CBG (Scotts/Monsanto 2015a).

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (US-FDA 2006) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984 1992). Scotts/Monsanto submitted a food/feed safety and nutritional assessment summary document for ASR368 CBG to the FDA in September 2002 and completed its consultation with the FDA, identified under BNF No. 000079, on September 23, 2003 (FDA 2003).

B. Development of ASR368 Creeping Bentgrass

The origin of creeping bentgrass (CBG) has been debated but most likely it originated in Eurasia (MacBryde 2006; Cook 2008). CBG is present in all states in the United States. According to some, it was probably introduced into North America prior to 1750 (Hannaway and Larson 2004). CBG is a fast growing perennial that reproduces by creeping stolons where plants are already established, and can also proliferate by seed (MacBryde 2006; Utah State University 2011). Seedlings can mature and set seed within the first growing season (Esser 1994). CBG is a cool-season turfgrass, mainly used on golf courses and other playing fields because of its ability for dense growth) and tolerance of close mowing (Cook 2008). It is also used for erosion control, cover, and food for wildlife and forage (Hannaway and Larson 2004; Cook 2008). When present in turf, CBG can produce large amounts of thatch (Utah State University 2011).

Glyphosate (*N*-(phosphonomethyl) glycine) is a broad-spectrum systemic herbicide that is highly effective against the majority of annual and perennial weeds common to grass seed and turf production (Scotts/Monsanto 2015a). It is registered with the Environmental Protection Agency (EPA) for nonselective weed control for both non-food use and food use plants (EPA 1993). Glyphosate's mode of action is to inhibit the enzyme 5-

enolpyruvylshikimate-3-phosphate synthase (EPSPS) involved in the metabolic pathway leading to the synthesis of the aromatic amino acids tyrosine, tryptophan and phenylalanine (Duke and Powles 2008). Glyphosate is structurally similar to the substrate for EPSPS and thereby competes with this substrate for the enzyme's active site, thus preventing the synthesis of aromatic amino acids and killing the plant.

ASR368 CBG was developed to enable the use of glyphosate herbicides for effective control of weeds occurring in the production of CBG seed and to maintain superior quality turf on golf courses (Scotts/Monsanto 2015a). In ASR368 CBG, metabolic requirements for plant growth and development are met by the continued action of the glyphosate resistant CP4 EPSPS enzyme in the presence of glyphosate (Funke et al. 2006; Duke and Powles 2008; Scotts/Monsanto 2015a)).

The CBG plant tissue that received DNA conferring resistance to glyphosate was embryogenic plant callus derived from a single seed of the CBG cultivar Backspin (cell line B99061R (Scotts/Monsanto 2015a). This plant and tissue was chosen for insertion of the *cp4 epsps* gene because it responds well to biolistic transformation and is successful in tissue culture regeneration.

ASR368 CBG was chosen from among more than four hundred transformation events based on agronomic and phenotypic characteristics and resistance to glyphosate. Using a forward breeding strategy, clones of the ASR368 CBG R_0 generation were crossed with a number of Elite Parent Plants (EPPs) to develop R_1, F_1 and F_2 progeny populations (Scotts/Monsanto 2015a). As a result, each individual plant of an ASR368 CBG seedling population is genotypically and phenotypically distinct yet representative of *A. stolonifera*.

To help establish greater familiarity with ASR368 CBG, and to better understand its plant pest and 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 examined the biology, morphology and life history of plants derived from ASR368 CBG. The results of these experiments are presented in Section VIII of the Petition (pp.118-247, Scotts/Monsanto 2015a). The stages of the life cycle and the plant characteristics evaluated at each stage that could contribute to ASR368 CBG posing a plant pest risk are provided in Figure VIII-1 of the Petition (pp.123, Scotts/Monsanto 2015a).

A number of different comparators were used to assess whether ASR368 CBG was altered in a biologically meaningful manner, including: (51 FR 23302) commercial cultivars that represent the range of *A. stolonifera* agronomic and phenotypic characteristics (Backspin, Penncross, Penn A-4, Crenshaw, and others), (2) Elite Parent Plants (EPPs), which were selected from commercially available *A. stolonifera* cultivars developed before 1994 and were crossed with ASR368 CBG R_0 generation plants to produce the R_1, F_1 and F_2 progeny populations, (51 FR 23302) null segregant or "Glyphosate Susceptible" (Stallings et al.) plants identified among segregating ASR368 CBG R_1 or F_1 progeny populations, from which non-transgenic populations were developed, and/or (57 FR 22984) "non-transgenic plants generated from the same

B99061R tissue culture line from which ASR368 CBG was derived (these non-transgenic plants are henceforth referred to as B99061R)" (Scotts/Monsanto 2015a; Harriman 2016). Conventional non-genetically engineered plants, such as the above, are generally are used as comparators for genetically engineered plants (EFSA 2011).

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of the insertion (e.g. nucleus or organelle) and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in ASR368 CBG relative to non-transgenic CBG. The assessment encompasses a consideration of the expressed enzyme (EPSPS) and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, anti-nutrients or nutrients in forage derived from the GE crop event compared to those in the conventional counterpart or to other comparators.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in the GE crop event, or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, non-target beneficial organisms, weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

ASR368 CBG was developed using biolistic transformation technology to deliver a 6700 base pair linear DNA segment derived from plasmid PV-ASGT08 containing two *cp4 epsps* gene expression cassettes to plant cells (Scotts/Monsanto 2015a). This DNA delivery system is well documented to transfer and integrate new DNA into a plant genome (Klein *et al*. 1987; Sanford *et al*. 1993; Lee 1996). After DNA delivery, cells were transferred to a selective media containing glyphosate and only those cells transformed with the *cp4 epsps* gene continued to grow. None of the inserted sequences from plant pests encode a plant pest or infectious agent. A complete description of the inserted genetic material can be found below ((Table V-1, pages 84-85, Scotts/Monsanto 2015a).

Summary of Genetic Elements in ASR368 CBG:

- Genomic DNA flanking the 5' end of the insert (page 97, Scotts/Monsanto 2015a).
- P-ract1/ract1 intron- The 5' region of rice (*Oryzae sativa*) actin1gene containing the promoter, transcription start site and first intron (McElroy et al. 1990), used to drive expression of the first *ctp2-cp4 epsps* cassette
- *ctp2*- The DNA sequence for chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS; the transit peptide directs the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid biosynthesis (Klee *et al*. 1987).
- *cp4 epsps*- The coding sequence for the 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium sp*. strain CP4 (Padgette *et al*. 1995).
- NOS 3'- The 3' untranslated region of the nopaline synthase (Honig *et al*.) gene from *Agrobacterium tumifaciens*, which terminates transcription and directs polyadenylation (Fraley et al. 1983).
- P-e35S- 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 second *ctp2-cp4 epsps* cassette.
- *Zm* HSP70 intron- The intron from the maize (*Zea mays*) *hsp70* gene (heat shock protein), used to stabilize the level of gene transcription (Rochester *et al*. 1986).
- *ctp2*- The DNA sequence for chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS.
- *cp4 epsps*- The coding sequence for the native 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium sp*. strain CP4
- NOS 3'- The 3' untranslated region of the nopaline synthase (Honig *et al*.) gene from *Agrobacterium tumifaciens*.
- Genomic DNA flanking the 3' end of the insert (page 98, Scotts/Monsanto 2015a).

Scotts/Monsanto provided evidence demonstrating that:

- the genome of event ASR368 CBG contains a single DNA insertion comprising a single copy of the DNA segment used for transformation (sectionsV.A.2.1.1-V.A.2.6 pp. 76-78, Scotts/Monsanto 2015a);
- both *cp4 epsps* gene expression cassettes within the single insert are intact; (sectionsV.A.2.1.1-V.A.2.6, V.A.2.8-V.A.2.10 pp. 76-80, Scotts/Monsanto 2015a);
- 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 (Honig *et al*. 2015) 3' polyadenylation sequence from *A. tumefaciens* (sectionsV.A.2.1.1-V.A.2.6, V.A.2.8-V.A.2.10 pp. 76-80, Scotts/Monsanto 2015a);
- 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 from Zea mays, CTP2 and the NOS 3' polyadenylation sequence (ections V.A.2.1.1-V.A.2.6, V.A.2.8-V.A.2.10 pp. 76-80, Scotts/Monsanto 2015a);

- the genome of event ASR368 CBG does not contain any detectable plasmid backbone DNA section (sectionsV.A.2.7 pp. 78-79, Scott/Monsanto 2015);
- the inserted DNA is stably inherited through the R_0, F_1 and F_2 generations of ASR368 CBG (section V.A.2.8. pp. 79, Scotts/Monsanto 2015a).

Scott/Monsanto examined the T-DNA insertion site in ASR368 CBG and corresponding conventional control line using Polymerase Chain Reaction (PCR) and sequence analyses and no unintended sequence rearrangements were found (Figures V3- V16 pp. 86-102, Scotts/Monsanto 2015a). The deduced amino acid sequences of the CTP-CP4 EPSPS proteins encoded in event ASR368 CBG were identical to the deduced amino sequences of the CTP-CP4 EPSPS proteins encoded in plasmid vector PV-ASGT08 (Scotts/Monsanto 2015a).

Segregation data from reciprocal crosses made between F_1 plants hemizygous for the *cp4 epsps* gene and elite parental plants indicate that a single T-DNA insert in event ASR368 CBG 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 (page 103, Scotts/Monsanto 2015a). These results are consistent with genomic analysis 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 CBG (pages 79 and 85, Scotts/Monsanto 2015a).

Expression of inserted DNA, changes in gene expression, new proteins or metabolism

The enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), catalyzes the synthesis of 5-enolpyruvylshikimate-3-phosphate (EPSP), one of the intermediates in the shikimic acid pathway (Levin and Sprinson 1964; Steinrücken and Amrhein 1980). EPSP is a precursor for the biosynthesis of the aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules necessary for plant growth. Genes for numerous EPSPS proteins have been cloned and the catalytic domains of this group of proteins are conserved. Bacterial EPSPS proteins have been well characterized with respect to their three dimensional structures (Stallings *et al*. 1991).

EPSPS is the target for the broad spectrum herbicide glyphosate, the active ingredient in Roundup agricultural herbicides (Steinrücken and Amrhein 1980; Herrmann and Weaver 1999). Thus, in conventional plants, glyphosate blocks the biosynthesis of EPSP thereby depriving plants of essential amino acids (Steinrücken and Amrhein 1980). Glyphosate resistance in ASR368 CBG comes from the expression of the bacterial (*Agrobacterium* sp. strain CP4) gene *cp4 epsps*. The *cp4 epsps* gene encodes CP4 EPSPS protein, which like plant EPSPS protein, catalyzes the synthesis of EPSP (Alibhai and Stallings 2001). The CP4 EPSPS protein is structurally similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate relative to endogenous plant EPSPS (Padgette *et al.,* 1996). Therefore, in genetically engineered glyphosate resistant plants containing the *cp4 epsps* gene, requirements for aromatic amino acids and other metabolites are met in the presence of glyphosate by the continued action of the CP4 EPSPS enzyme (Padgette *et al*. 1996).

In ASR368 CBG the *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (page 68, Padgette *et al*. 1996; Scotts/Monsanto 2015a). CP4 EPSPS protein produced in ASR368 CBG was demonstrated to be equivalent to both *E. coli*- produced CP4 EPSPS, used previously for human and animal safety studies, and CP4 EPSPS protein produced in commercial Roundup Ready soybean (Monsanto event 40-3-2: approved petition #06-178-01p) based on identical electrophoretic mobilities and detection using specific antibodies as established by western blot analysis (Figure VI-1 pp. 107, Scotts/Monsanto 2015a). This demonstration of equivalence justifies the use of previously conducted protein studies using CP4 EPSPS protein from *E. coli* to establish the safety of the CP4 EPSPS protein expressed in ASR368 CBG (Section V1.B pp. 106-107, Scotts/Monsanto 2015a).

The history of safe use of CP4 EPSPS has been previously reviewed as a part of the safety assessment of this protein for USDA-APHIS determinations of nonregulated status (USDA-APHIS-BRS 1994, 1995, 2004, 2010, 2012, 2013, 2014), as well as completed consultations with the FDA (FDA 2003). EPA has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production in all plants (US-EPA 2007). Additionally, Scotts/Monsanto (2015a) reported that their bioinformatics studies show that the CP4 EPSPS protein does not share amino acid sequence similarities with known allergens and, therefore, is highly unlikely to contain immunologically cross-reactive allergenic epitopes. FDA (FDA 2003) (2003) supported Scotts/Monsanto's contention that the EPSPS is safe in FDA Biotechnology Consultation Note to the File BNF No. 000079.

Levels of the CP4 EPSPS protein were estimated in event ASR368 CBG forage samples collected from four replicated field sites during the 2000 - 2001 growing season (Table VI-1; pp. 109, Scotts/Monsanto 2015a). Field production was conducted using agronomic practices typical of the commercial cultivation of CBG. The field sites were representative of geographical regions where CBG could be grown under the appropriate environmental conditions. The samples were analyzed using as enzyme- linked immunosorbent assay (ELISA) (Harlow and Lane 1988) to estimate the level of CP4 EPSPS protein present in forage tissue over five time points : ~100 days after planting, ~330 days after planting, ~390 days after planting and ~480 days after planting (Scotts/Monsanto 2015a). Forage samples consisted of the whole aerial portion of the plant and were harvested at the late vegetative growth (pseudo-erect) stage. The grand average CP4 EPSPS protein level in ASR368 CBG forage across all time points was 68.6 µg/g fresh weight of tissue (fwt), with a standard deviation of 16.9 µg/g fwt (Scotts/Monsanto 2015a). The levels showed little change over time, demonstrating the stability of expression of the CP4 EPSPS protein.

Comparative compositional analyses were conducted on leaf forage samples from ASR368 CBG, B99061R, and three conventional varieties produced in replicated plots established at four replicated field sites during the 2000-2001 growing season. Single samples of four additional conventional varieties were also included to establish commercial ranges and 99% tolerance intervals to provide additional information on the range of natural variability for each component. McCrimmon (1994)

and Youngberg and Vough (1977) were consulted to determine the appropriate compositional analytes and their range in CBG 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 CBG.

In a combined-site analysis in which the data were pooled among the sites, there were no statistically significant differences observed between ASR368 CBG and the control B99061R for any of the analytical components (Scotts/Monsanto 2015a). In an individual-site analysis of the data, four statistically significant differences were observed between ASR368 CBG and B99061R among three different analytical components (Table VII-2 pp. 117, Scotts/Monsanto 2015a). Statistically significant differences were detected for the content of moisture (1 site), phosphorus (1 site), and NDF (2 sites). Of the four comparisons observed to be statistically different between ASR368 CBG and B99061R, all values of ASR368 CBG were within the range and 99% tolerance interval of the conventional, commercial varieties (Scotts/Monsanto 2015a). The significant differences were only observed at one or two sites, not in the combination of all the field sites, and are not considered to be biologically meaningful.

Based on the above analyses, APHIS concludes that the genome of event ASR368 CBG contains a stable, single DNA insertion and both *cp4 epsps* gene expression cassettes are intact. Other than the expression of CP4 EPSPS protein, there are no biologically meaningful differences in the composition of ASR368 CBG compared to non-genetically engineered CBG. CP4 EPSPS protein has been evaluated and determined to be safe in various genetically engineered (GE) crops. In addition, FDA (2003) concluded that ASR368 CBG is "is not materially different in composition, safety, or any other relevant parameter from conventional creeping bentgrass other than it's tolerance to glyphosate."

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in ASR368 CBG (as identified from the previous section) that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses. APHIS also assessed or whether ASR368 CBG is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would 1) affect the new GE crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or 3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease

susceptibility was evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (USDA-APHIS-PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America, and that supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (USDA-APHIS-BRS 2013) however none specifically target pests of the ASR368 CBG.

No plant pest transformation vectors were used in the creation of ASR368 CBG since transformation was accomplished through biolistics. Although genetic material from plant pests was inserted into the ASR368 CBG genome, none of the inserted sequences from plant pests encode a plant pest or infectious agent. Field releases of ASR368 CBG were conducted in which researchers monitored field sites for disease and pest susceptibility. The diseases and insects monitored during these field trials are summarized in Table VIII-112 of the Petition (pp. 238-240, Scotts/Monsanto 2015a). Observations of plant growth and disease and insect pest susceptibility of ASR368 CBG were documented for 65 field releases in 20 states and 40 counties performed between 1999 and 2002 (Scotts/Monsanto 2015a). Major turf diseases that were monitored included: dollar spot (*Sclerotinia homocarpa*), brown patch (*Rhizoctonia solani*), snow mold (*Myriosclerotinia borealis, Typhula incarnata*), leaf spot (*Helminthosporium sp., Dreschelera* sp., *Septoria* sp.), take-all patch (*Gaeumannomyces graminis*), copper spot (*Gloeocercospora sorghil*), leaf rust (*Puccinia* sp.), spring dead spot (*Leptosphaeria narmari*), and *Pythium* (*Pythium* sp) . Major insect pests that were monitored included: 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.).) Visual observations were made while walking the fields and, in almost all circumstances, the observations were qualitative rather than quantitative.

After reviewing the information gathered over three years of monitoring ASR368 CBG, there were no discernible differences in disease severity/insect infestation between ASR368 CBG and conventional CBG plants (Table VIII-12, pp. 283-240, Scotts/Monsanto 2015a). There were also no differences in plant growth or agronomic characteristics that might be indicative of increased disease severity or insect infestation in ASR368 CBG as compared to conventional plants in these trials (Scotts/Monsanto 2015a). 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 (Scotts/Monsanto 2015a).

As discussed earlier there were no significant changes in ASR368 CBG composition that would render ASR368 CBG more susceptible to pests and diseases over its control or reference CBG varieties. The observed agronomic traits also did not reveal any significant changes that would indirectly indicate that ASR368 CBG is or could be more susceptible to pests and diseases compared to control or reference CBG varieties.

Considering the results of the agronomic, compositional, disease and insect evaluations, ASR368 CBG is unlikely to be more susceptible to plant pathogens and insect pests than conventional CBG. For this reason, ASR368 CBG is unlikely to differ from conventional CBG in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Non-Target Organisms Beneficial to Agriculture

ASR368 CBG is not engineered for pest resistance. Thus there are no 'target' or 'non-target' species. APHIS assessed whether exposure to or consumption of ASR368 CBG would have a direct or indirect adverse impact on species beneficial to agriculture**.** The assessment includes an analysis of data and information on ASR368 CBG compared to the non-GE counterpart (or other comparators) for any biologically relevant changes in the phenotype or substances (e.g. proteins, nutrients, metabolites) produced which may be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture.

Scotts/Monsanto and other sources provided the following information about the effect of CP4 EPSPS on beneficial organisms (Section V.D., pp. 72-75, Scotts/Monsanto 2015a):

- CP4 EPSPS protein is homologous to EPSPS proteins naturally present in plants (Section VI.E.2 pp.111, Scotts/Monsanto 2015a) including food crops (e.g., soybean and maize) and fungal and microbial food sources such as baker's yeast (*Saccharomyces cerevisiae*) (Harrison *et al*. 1996; Padgette *et al*. 1996).
- CP4 EPSPS protein has been evaluated and determined to be safe in glyphosate resistant products across several crops including soybean, corn, canola, cotton, and sugar beet (USDA-APHIS-BRS 1994, 1995, 2004, 2010, 2012, 2013, 2014)
- An acute oral toxicology study with mice indicated that the CP4 EPSPS protein did not cause any adverse effects in mice at the highest dose level tested (572 mg/kg) (Harrison *et al*. 1996). Harrison *et al.* stated that "CP4 EPSPS is rapidly degraded in mammalian digestive systems, reducing exposure, and has no significant sequence or structural homology to known toxins or allergens."
- The results of a 1996 study that tested consumption of the CP4 EPSPS protein by rats, chickens, catfish and dairy cattle confirmed the results of other studies that demonstrated the safety of the introduced protein (Hammond and Vicini 1996).

- Field observations were made on the incidence of beneficial organisms (lady beetles, spiders, and honeybees) while walking the fields and, in almost all circumstances, the observations were qualitative rather than quantitative. Observations from multi-year U.S. field trials of ASR368 CBG and progeny support conclusions of no adverse impacts of glyphosate resistant creeping bentgrass on beneficial organisms.

In previous determinations, USDA-APHIS has made determinations of nonregulated status for EPSPS glyphosate-resistant events in corn (MON 87411, MON 88017, MON 87427, NK603, GA21), cotton (MON88913,), canola (MON88302,), soybean (GTS 40-3-2, MON89788), sugarbeet and alfalfa (J101, J163) (https://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml). In each of these events, an analysis of the impact to beneficial organisms was conducted without identifying a negative effect of the CP4 EPSPS protein. The EPSPS proteins have been commercialized since 1994 and are present in crops which are grown on millions of acres in the U.S. every year. At present, APHIS is not aware of any identified significant adverse effects of EPSPS proteins on beneficial organisms in the field.

Therefore, based on the above analysis of the safety of the protein CP4 EPSPS, observations from multi- year U.S. field trials looking for adverse non-target interactions with the use of ASR368 CBG and evaluations of the impact of the EPSPS protein in previous determinations of nonregulated status, APHIS concludes that exposure to and/or consumption of ASR368 CBG and the CP4 EPSPS protein are unlikely to have any adverse impacts on non-target organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of ASR368 Creeping Bentgrass

APHIS assessed whether ASR368 CBG is likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the non-transgenic CBG progenitor from which it was derived, or other varieties of CBG currently being grown. The assessment considers the basic biology of CBG, the situations in which CBG populations are considered weeds, and an evaluation of ASR368 CBG compared to the non-transgenic progenitor and other appropriate counterparts evaluated under field (and/or lab) conditions characteristic for the regions of the United States where ASR368 CBG was intended to be grown. Characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage CBG as a weed were evaluated. For CBG, such characteristics include seedling and vegetative propagule establishment, growth rate, competitiveness, flowering and pollen characteristics, fecundity, seed dormancy and germination, and seedling vigor. The assessment also considers whether the engineered trait affects methods of control for CBG in situations where it is managed as a weed or volunteer.

Conventional CBG

Creeping bentgrass is a phenotypically plastic and evolutionarily adaptive species that has naturalized across the United States (Banks *et al*. 2004; MacBryde 2006; BONAP 2013; USDA-NRCS 2016g), although there is uncertainty regarding whether some northern populations may be native (Banks *et al*. 2004; Harvey 2007; NatureServ 2015). It is an important grass on golf greens around the world, is used as a moderately productive forage, can be helpful in preventing soil erosion, and serves as cover and food for various wildlife (Esser 1994; Hannaway and Larson 2004). CBG is generally found in moist, often disturbed areas with low environmental stress, including moist meadows, pastures, hayfields, and forest edges, coastal scrub and beaches, the banks and edges of lakes, ponds, marshes, rivers, streams, creeks, canals and ditches, in home lawns and recreation areas, and along roadsides, railroad rights-of-way and in waste lands (Widén 1971; Wiens 1984; Hunt *et al*. 1987; Kik *et al*. 1990a; Edgar and Forde 1991; Banks *et al*. 2004; Harvey 2007; Ahrens *et al*. 2011a; Bollman *et al*. 2012). Wetlands and riparian areas with intermediate management or disturbance regimes provide the best habitat for CBG, though it can grow less well in drier areas or habitats that impose more stress (Widén 1971; Edgar and Forde 1991; Banks *et al*. 2004; Ahrens *et al*. 2011a; Scotts/Monsanto 2015a).

The Pacific northwest U.S. is the primary location of grass seed production in the U.S. Feral CBG is reported in most counties in Idaho, Oregon, and Washington State (BONAP 2013). In eastern Oregon and western Idaho, where ASR368 CBG has escaped cultivation (Zapiola *et al*. 2008; Scotts/Monsanto 2015a), rainfall levels are low and, as expected based on its moisture requirements, feral CBG grows primarily along creeks and streams, irrigation canals and ditches, and in other ditches, springs, and ponds (Watrud *et al*. 2004; Zapiola *et al*. 2008; Bollman *et al*. 2012; Scotts/Monsanto 2015a; Consortium of Pacific Northwest Herbaria 2016).

Establishment, Persistence, and Spread

CBG can establish from seed or from vegetative stolons, in relatively open areas and bare soil as well as in areas that may be somewhat densely vegetated (Eriksson 1989; Jónsdóttir 1991; Banks *et al*. 2004; MacBryde 2006). However, seedling establishment generally appears to require disturbed or bare soils, since it is generally infrequent in established vegetation, where local spread occurs primarily through clonal expansion of established colonies via stolons (Kik *et al*. 1990b; Jónsdóttir 1991; Bullock *et al*. 1994; Hoeltzener and Maitre 2004; Jones 2011). Thus, new seedlings do not contribute substantially to stand augmentation in established vegetation, but rather, grow most frequently in the margins or where disturbances of existing plants occur (Reichman *et al*. 2006; Scotts/Monsanto 2015a). Establishment in new areas from vegetative fragments may also require disturbance, as CBG plants transplanted into existing vegetation generally do not survive well without disturbance or regular management (Garrison and Stier 2010; Aherns and Auer 2012). However, once established, CBG resists competition and colonies can persist in mixed grass swards (Lush 1988a; Scotts/Monsanto 2015a). In areas of high disturbance but high environmental stress, there is more reliance on sexual reproduction and the seed bank for survival than on vegetative mechanisms (Kik *et al*. 1990b).

Depending on habitat and management, CBG can be competitive and become a dominant, for example in moderately grazed pastures and various habitats of roadsides (MacBryde 2006; Ahrens *et al*. 2011b). It was able to expand over three years when transplanted into residential Kentucky bluegrass subject to regular mowing and into a mixed roadside turf that was first treated with herbicide and then subject to regular mowing (Hart *et al*. 2009). Due to its vigorous stolon growth, it can spread rapidly, grow over the top of other grasses, produce a dense, prostrate growing stand or sward, and form continuous mats in favorable habitats (Widén 1971; Dore 1980; Cal-IPC 2006; MacBryde 2006; Reicher *et al*. 2006; Cook 2008).

However, CBG tends to survive best in areas with rather open plant cover and weak competition from other plants, though it can also withstand some shading (Widén 1971; Esser 1994). Thus, it ultimately may decline over years or decades, as in maturing sand dunes, hay meadows, or old golfing greens (Esser 1994; Cook 2008). For instance, in a study of two abandoned golf courses, CBG was nearly absent from one within five years after operations ceased, while the other contained less than 25% CBG only two years after maintenance stopped (Garrison *et al*. 2009). Annual bluegrass (*Poa annua*) is able to outcompete CBG and, if left uncontrolled, will ultimately take over most old golf greens (Cook 2008). CBG did not compete well when transplanted into existing vegetation in prairie, meadows, hayfields, and wasteland that were subject to minimal or no management practices (Garrison and Stier 2010; Aherns and Auer 2012). Nonetheless, CBG persists in favorable habitats such as riparian areas, overgrazed pastures and meadows, and roadsides. A large scale study in Connecticut found that persistence of established populations was positively correlated with soil moisture and mowing, and negatively correlated with tree or shrub cover, poorly drained soils, and leaf litter (Ahrens *et al*. 2011a).

There are three primary methods by which CBG can spread: seeds, roots and stolons. As noted above, CBG spreads vegetatively by stolons, and both the roots and the stolons actively search for areas where there may be regions of nutrients and vegetation breaks (MacBryde 2006and references therein). In addition, stolon fragments can regenerate to produce separate plants that are capable of establishing (Widén 1971; Scotts/Monsanto 2015a). Jointed pieces of the stolons (*i.e.* with nodes) and seeds can be carried downstream by water, vehicles, and shoes to new areas of establishment (Banks *et al.*, 2004; MacBryde, 2006 and references therein)

CBG also produces numerous tiny seeds that are dispersed by the wind, water (Wolters *et al*. 2005), and sometimes by cattle, sheep, white-tailed deer, fallow deer and geese (Welch 1985; Gill and Beardall 2001; Chang *et al*. 2005). Zapiola and Mallory-Smith (2012) reported that CBG panicles that fall in an irrigation canal have the potential to travel downstream at an average rate of 19 m min^{-1} and move seeds that could potentially establish seedlings elsewhere. The seeds did not lose their ability to germinate after 17 weeks in water at 20^{o}C and germination was still 46% after 17 weeks in water at 4^{o}C. The reduction in germination in seeds from panicles kept in water at 4^{o}C was due to the induction of secondary dormancy, which was overcome by dry seed storage at room

temperature. Seeds can germinate soon after their dispersal, or they can remain dormant for up to at least four years in the seed bank (Thompson and Grime 1979; Shipley *et al*. 1989; Shipley *et al*. 1991; Ferris and Simmons 2000; Mitlacher *et al*. 2002; Wolters and Bakker 2002; Díaz-Villa *et al*. 2003; Hölzel and Otte 2004; MacBryde 2006).

CBG is mainly sexually outcrossing (Belanger *et al*. 2003b). In experimental field-plot studies on pollen dispersal from small groups of CBG plants, viable pollen moved as far as 1,161 feet, with the amount of pollen movement decreasing rapidly with increasing distance (Wipff and Fricker 2001a; Christoffer 2003; Belanger *et al*. 2003b).

Weed Status and Control

As demonstrated above, CBG has many traits associated with weediness such as its wide adaptability, ready dispersal, regeneration abilities after damage, and seed dormancy. Seeds and stolons pieces of CBG can spread via waterways and the seeds can remain in seedbanks until germination or remain dormant for several years. Spread of CBG in older stands is mostly by stolons/roots with seed dispersal being the route of establishment in new areas.

These and other weediness traits resulted in high weed risk scores in weed risk assessments of CBG conducted by APHIS-PPQ and the Pacific Island Ecosystems at Risk project (Pacific Island Ecosystems at Risk (PIER) 2011; USDA-APHIS-PPQ 2014a). The California Invasive Plant Council (Cal-IPC) and NatureServe found that it can form thick mats or dense swards in some habitats, which may change community composition (Cal-IPC 2006; NatureServ 2015). Bollman *et al* (2012) argue that CBG is an invasive species in wetland and riparian habitats. According to National Park Service surveys, CBG is invasive in some United States National Parks (Swearingen 2009; Invasive Plant Atlas of the United States 2015). It's also listed as a weed in Weeds of Nebraska and the Great Plains (USDA-NRCS 2016c) and Weeds of the West (Burrill *et al*. 1999; USDA-NRCS 2016d).

However, even though CBG possesses weediness traits, it is not generally considered a particularly troublesome weed. It has not been listed as an important weed in the U. S. in major weed references (Muenscher 1980; USDA-NRCS 2016a, 2016b) or Federal or State noxious weed lists. The California IPC and NatureServe found that it has only a minor or low impact, respectively, on native ecosystems (Cal-IPC 2006; NatureServ 2015). APHIS-PPQ found that despite its high weed risk score, CBG is unlikely to have much impact as a weed in agricultural systems other than potentially affecting yields and quality of grass seed crops (USDA-APHIS-PPQ 2014a).

In a comprehensive literature review and survey of over 90 weed scientists and other experts, Banks *et al.* (2004) found that where CBG and other bentgrass species occur "they are relatively non-aggressive, their presence is rarely considered a problem that warrants management and thus they are generally not managed as weeds." The only settings in which they sometimes pose an important problem as weeds are in other turfgrasses and in grass seed fields, where their importance as weeds was ranked as high and moderate respectively (Banks *et al*. 2004). There have been some reports of CBG

being an occasional weed of low importance in fruit crops, landscapes and ornamentals, pastures and rangelands (generally only in riparian areas), roadsides, and natural areas, but they were not identified as important, significant, or problem weeds in any of these environments (Banks *et al*. 2004; Ahrens *et al*. 2011b). However, its persistence in riparian habitats, particularly irrigation canals and ditches, and in other disturbed areas provides potential sources for introduction of CBG into grass turf and seed production fields.

A primary herbicide used to eliminate CBG where it is not desired is glyphosate (Banks *et al*. 2004). It is the herbicide of choice in the riparian areas. However as discussed further below, other herbicide options are available.

Glyphosate-resistant CBG

APHIS assessed whether the weed potential (prevalence, competitiveness, damage) of ASR368 CBG or glyphosate resistant CBG (GRCBG) derived from it could be greater than that of non-herbicide resistant CBG. APHIS also assessed whether the engineered trait could render GRCBG more difficult to control in situations where CBG is managed as a weed.

Establishment, Persistence, and Spread

Scotts/Monsanto (2015a) provided data and information comparing ASR368 CBG to non-transgenic CBG for various characteristics related to weediness (establishment, competitiveness, dormancy, germination, etc.). Some of this data has also been published elsewhere (Gardner *et al*. 2003; Fei and Nelson 2004; Gardner *et al*. 2004).

Establishment

- Seedling establishment studies were conducted from 2000 to 2002 to compare the ability of ASR368 CBG and four non-transgenic cultivars (Backspin, Crenshaw, Penn A-4, Penncross) to establish from seed under both irrigated and non-irrigated conditions, in the presence or absence of competition from other grasses, and in different seasons. These studies were conducted in Marion County, Oregon during 2000, 2001 and 2002, and Franklin County, Massachusetts during 2000 – 2001 (Section VIIIA, Scotts/Monsanto 2015a). Marion County is located in the Willamette Valley and receives substantially more rainfall than the counties west of the Sierra Mountains where ASR368 CBG has escaped (WRCC 2016). In addition, the seed planting dates in Oregon were timed to coincide with the approximate start of the rainy winter season in order to optimize germination and establishment under natural conditions.

  Establishment of ASR368 CBG and all non-transgenic comparators seeded in bare soil was generally low, never exceeding 30% seedling survival. None of the varieties were able to establish when seeded into mature vegetative stands of other grasses (Tables VIII-7 to VIII-9 and section VIII.A.2.2.4,Scotts/Monsanto 2015a), and they had poor or no survival when

seeded on bare ground in the spring in Marion County, OR, even when irrigated (Table VIII-5 and section VIII.A.2.2.3). All of the varieties were able to germinate and survive when seeded on bare ground in the fall in Marion County, with substantially greater survival when irrigated during the germination and initial establishment phase. However, both when irrigated (fall 2001) and not (fall 2000), ASR368 CBG had consistently lower survival than one variety, and no consistent differences in survival than the other varieties (Tables VIII-4 and VIII-11). ASR368 CBG did exhibit consistently greater survival in the absence of irrigation than one of the four non-transgenic varieties between 7 and 12 months after the fall 2001 planting (Table VIII-12). Taken as a whole, these results are consistent with the known requirements for CBG establishment. Although the experiments used relatively few seeds, the results indicate that ASR368 CBG is similar to conventional CBG in its ability to establish from seed in competitive and non-competitive ecosystems.

- Vegetative establishment studies were conducted in 2001 and 2002 to compare the establishment ability of detached stolon sections containing nodes from plants of glyphosate resistant (GR) ASR368 CBG R1 and F2 progeny, ASR368 glyphosate susceptible segregants (Stallings et al.) of ASR368 CBG, and six commercial cultivars (Backspin, Crenshaw, Penn A-4, Penncross, Penneagle, SR-1020) under controlled conditions (Section VIII.B.1, Scotts/Monsanto 2015a). Two concurrent studies were conducted: 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. In both experiments, stolons were maintained to prevent moisture stress. In the first experiment, GR ASR368 CBG stolons demonstrated significantly greater vegetative establishment than stolons of GS segregants and one conventional cultivar, and nearly significantly greater establishment than stolons of a second. However, GR ASR368 CBG stolons did not exhibit significantly greater establishment than stolons of two other conventional cultivars in this experiment, and there was no significant difference between GR ASR368 CBG another two conventional cultivars in the second experiment.

  Additional studies were performed during 2002-2003 to assess the ability of ASR368 CBG F1 and F2 progeny and four conventional cultivars (Backspin, Crenshaw, Penn A-4, Penncross) to vegetatively establish under field conditions in Marion County, Oregon; Union County, Ohio; Baldwin County, Alabama and in the greenhouse in Fayette County, Kentucky (Section VIII.B.2, Scotts/Monsanto 2015a). At each site the study was performed under both "irrigated" (designed to mimic a golf course) and "non- irrigated" (designed to mimic unmanaged ecosystems) conditions. Under irrigated conditions there was no significant difference in the ability of ASR368 CBG and conventional CBG stolons to produce tillers at any location except Marion County, OR, where stolons from ASR368 CBG F1 and F2 plants consistently produced tillers at a lower rate than stolons from one conventional cultivar, while F1 but not F2 plants tended to produce tillers at a higher rate than the other cultivars, although there were no consistent statistically significant differences. Under non-irrigated conditions there were no statistically significant difference between ASR368

CBG and the other cultivars. Moreover, consistent with the known requirements for CBG establishment, tiller production from stolons was poor to non-existent in all locations except Kentucky. The vegetative establishment experiments conducted during 2002 - 2003 further confirm that the potential for vegetative establishment of plants derived from ASR368 CBG is not significantly different from that of several accepted conventional cultivars.

Competiveness

- A number of experiments were performed in 2000, 2001 and 2002 comparing the relative growth of ASR368 CBG to B99061R and several conventional cultivars (Backspin, Crenshaw, Penn A-4, Penncross) and three other bentgrass species ('Highland' dryland bentgrass (*A. castellana*), 'SR7100' colonial bentgrass (*A. capillaris*) and 'Streaker' redtop bentgrass (*A. gigantea*)) in bare soil and in competitive managed turfgrass stands (Section VIII.C, Scotts/Monsanto 2015a). These experiments were conducted at eight locations representing several distinct environments: (51 FR 23302) cool season, (2) transitional climate, (51 FR 23302) warm season full sun, (57 FR 22984) warm season shade and (5) cool season reduced irradiance.

- In non-competitive cool season and transitional zone growth experiments of plants grown from stolon nodes in bare soil without mowing and managed with standard agronomic practices, there were no consistent differences between the growth of ASR368 CBG, B99061R, or three conventional cultivars, based on measurements of stolon length, percentage ground cover, and shoot density (Section VIII.C.1, Scotts/Monsanto 2015a). At some sites and some times in the year after planting ASR368 CBG showed more growth than one conventional cultivar and less growth than one or two other conventional cultivars. In a separate set of experiments, mature ASR368 CBG F1 plants planted into bare soil and F2 plants produced significantly less vegetative biomass than two of three conventional cultivars (Table VIII-83). ASR368 CBG glyphosate resistant R1 segregants also produced significantly less vegetative biomass than glyphosate sensitive R1 segregants (Scotts/Monsanto 2015a) (Table VIII-78). Finally, in non-competitive growth experiments conducted indoors under 17% - 20% normal irradiance, growth of ASR368 CBG was, with occasional and inconsistent exceptions, not statistically different from and within the range of B99061R and four conventional CBG cultivars (Section VIII.C.2.3Scotts/Monsanto 2015a) (Section VIII.C.2.3).

- In the competitive growth experiments, turf stands to be inter-planted with bentgrass were maintained for uniform turf coverage and surface drainage. After bentgrass plugs were transplanted into the turf and allowed to establish, the turf was maintained under a management regime appropriate to the original dominant species in order to maintain the existing turf (cool season and intermediate zones) or prevent moisture stress (warm season). In the cool season and transition zone experiments the turf was regularly mowed; Scotts/Monsanto

(2015a) did not indicate whether regular mowing occurred in the warm season experiments.

In the cool season and transition zone experiments (Section VIII.C.2.1, Scotts/Monsanto 2015a), the mean plant diameter of ASR368 CBG plants tended to fall within the range of the conventional cultivars at each of four locations and statistically significant differences were generally occasional and inconsistent. However, ASR368 CBG plants had a consistently larger mean diameter than B99601R plants at two of four sites. Also, in a cool season experiment in Marion County, Oregon planted in June 2000, ASR368 CBG plants had a consistently (though not always statistically significant) larger mean diameter than all three conventional cultivars in the first three months of 2001, and one of the cultivars for most of 2001, but thereafter had a consistently (though not always statistically significant) smaller mean diameter than all of the cultivars for the remainder of the experiment through early 2003.

In the warm season experiments (Section VIII.C.2.2, Scotts/Monsanto 2015a), plants were grown in either shade or full sun. Among plants grown in shade, ASR368 CBG plants were consistently larger than B99061R in the second year after planting, consistently and statistically smaller than one conventional cultivar, and generally but not statistically smaller than three other conventional cultivars. Among plants grown in full sun, ASR368 CBG plants were also consistently larger than B99061R in the second year after planting, but generally the same size or smaller (sometimes statistically so) than the conventional cultivars.

- Although in the competitive growth experiments ASR368 CBG often outperformed B99061R, the results of the non-competitive and competitive growth experiments taken as a whole indicate that ASR368 CBG displayed no increase in vegetative growth or relative fitness compared to conventional CBG cultivars. Thus, ASR368 CBG is unlikely to be any more competitive than conventional CBG cultivars.

Flowering, Pollen Characteristics, and Fecundity

- CBG plants that set flower earlier in the year or have a longer flowering duration may have enhanced reproductive potential. Scotts/Monsanto (2015a) assessed the flowering characteristics of ASR368 CBG in both greenhouse and field experiments (Section VIII.D). In greenhouse studies conducted over two years, ASR368 CBG R0 plants exhibited a statistically or nearly statistically faster time to flowering than one conventional cultivar in both years (2 days and 10 days). The F1 and F2 plants also had a consistently faster time to flowering than this cultivar in one of two years (8 and 10 days respectively). However, no other consistent and statistically significant differences in time to flowering were observed between R0, F1 and F2 plants compared to B99061R or two other cultivars. Conversely, in field studies ASR368 CBG F2 plants exhibited a statistically slower average time to flowering than two

conventional cultivars (1 day) but not a third. There were no statistically significant differences in time to flowering between F1 plants and the other cultivars. Flowering duration in the greenhouse studies was statistically or nearly statistically longer in ASR368 CBG F1 plants (but not R0 or F2 plants) than in B99061R and two of three conventional cultivars, by 3 – 5 days, in one of two years, but there were no significant differences in the second year. However, in the field studies there were no significant differences in flowering duration between ASR368 CBG F1 or F2 plants and the three cultivars. Overall, these results indicate that ASR368 CBG is unlikely to exhibit enhanced reproductive potential relative to non-transgenic CBG due to changes in flowering initiation or duration.

- No differences in pollen size, viability or longevity were observed between ASR368 CBG pollen and pollen from other CBG cultivars, indicating that pollen from ASR368 CBG is unlikely to differ from non-transgenic CBG in its ability to disperse, pollinate, or fertilize other sexually compatible CBG present in the environment (Tables VII-66 to VII-72, Scotts/Monsanto 2015a).

- A decrease or increase in seed production could affect a plant's ability to form a persisting population and consequently its weediness. Scotts/Monsanto assessed seed production by ASR368 CBG in both greenhouse and field experiments (Section VIII.F, Scotts/Monsanto 2015a). In greenhouse studies, the number of germinable seed and mature seed per panicle were evaluated under both open- and self- pollination conditions. Under open-pollination conditions, there was wide variation in the number of germinable seed per panicle and number mature seed per panicle between plants of the same genotype, with no statistically significant differences between ASR368 CBG and either B99061R or three conventional cultivars. The number of germinable seed produced upon self-pollination was very low for all CBG plants tested. This precluded detection of any differences between ASR368 CBG and conventional cultivars but was consistent with the nearly obligate outcrossing nature of CBG. In open-pollinated field studies, there were no statistically significant differences between GR and GS ASR368 CBG R1 segregants in the number of seeds per five panicles or seed mass. There were also no significant differences in seed per five panicles between ASR368 CBG F1 plants and three conventional cultivars. ASR368 CBG F2 plants had significantly more seed per five panicles than two of three conventional cultivars. However, on a whole plant basis, the number of seed per plant in ASR368 CBG F2 plants was significantly higher than only one of the three conventional cultivars. Moreover, the seed mass produced by both F1 and F2 plants was significantly lower than the seed mass of all three conventional cultivars. Lower seed mass may result in lower establishment rate and competitive ability (Whalley *et al.* 1966), thus counteracting the increase in seed number per plant in ASR368 CBG F2 plants relative to one of the three conventional cultivars. Overall, the results indicate that seed production is highly variable in CBG plants and that seed production by ASR368 CBG is within the range of seed production of conventional cultivars that are representative of CBG.

Dormancy, germination and seedling vigor

- Seed viability, dormancy and longevity of ASR368 CBG R1 seed, and the vigor of germinated seedlings, were evaluated relative to that of seed and seedlings from the conventional creeping bentgrass variety SR1020 using four tests: (51 FR 23302) standard germination test (SGT); (2) suboptimal temperature test (SUB); (51 FR 23302) supra-optimal temperature test (SuOP) and (57 FR 22984) accelerated aging test (AAT) (Section VIII-G, Scotts/Monsanto 2015a). Each test was conducted with four replications each of 100 seeds of the test genotypes. Germination percentage was recorded each week for four weeks. At the end of each test period (28 days), plants were moved to a greenhouse at 24°C ± 6°C and continuous light. During the first two weeks in the greenhouse, 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. Additionally after being classified into high or low germination energy and seedling vigor, plants of each ASR368 CBG R1 seed lot were sprayed with glyphosate to determine the percentage that were glyphosate resistant (GR) or sensitive (Stallings et al.).

- AOSA Standard Germination Test (SGT): Total germination percentage was used as an indicator of seed-lot quality and to provide a baseline for the ratio of GR to GS progeny to expect under ideal conditions. The percentage 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. Seed samples were evaluated for viability using the standard germination test as described in the AOSA Rules for Testing Seeds (1999). Neither the percentage germination (Table VIII-88Scotts/Monsanto 2015a) nor the germination rate (Scotts/Monsanto 2015a) (Table VIII-91) demonstrated a statistically significant difference between seed lots derived from ASR368 CBG R1 and SR1020. The germination percentages for these cultivars were 87.8% and 89.8% respectively. The total percentage of GR plants among ASR368 CBG R1 seedlings was 49.2% (Table VIII-89), which is consistent with the expected 1:1 segregation of GR to GS seeds within the R1 seed lots produced from the R0 primary transformant of event ASR368. Similarly, the percentage of GR plants among seedlings with high germination energy and seedling vigor (49.7%) was not significantly different from the percentage of GR plants among seedlings with lower germination energy and seedling vigor (49.1%) (Table VIII-90). Therefore, seed derived from ASR368 CBG is no more likely to germinate, and is unlikely to establish faster or be more competitive, than seed that does not possess the glyphosate resistance trait.

- 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 15ºC to 24ºC for cool season turfgrass growth. 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 GR to GS increases significantly under varying environmental stresses compared to the ratio under SGT, then the transgene likely imparts a selective advantage for germination under stress.

  In the SUB test the percentage of ASR368 CBG R1 seed that germinated did not differ significantly from that of the conventional bentgrass cultivar SR 1020 (Table VIII-88). The percentage of GR seedlings recovered (51.1%) was not significantly different from the percentage of GR seedlings recovered under SGT conditions (Table VIII-89). In addition, the percentage of seedlings with high germination energy and seedling vigor that were GR (52.45%) was not significantly different from the percentage of seedlings with lower germination energy and seedling vigor that were GR (50.52%) or from the corresponding percentages in the SGT test. Therefore, seed derived from ASR368 CBG is no more likely to germinate or be more competitive than seed that does not possess the glyphosate resistance trait under sub-optimal temperatures.

In the SuOP test, the germination percentage of ASR368 CBG R1 seed was significantly lower than the germination percentage of SR1020 (81.5% vs 90.3% respectively, (Table VIII-88, Scotts/Monsanto 2015a) did not conduct tests to assess the viability of non-germinated seed. Thus, this result may indicate either that ASR368 CBG R1 seed have decreased viability relative to SR1020 seed following exposure to high temperatures or that ASR368 CBG R1 seed have increased temperature induced dormancy than SR1020 seed. Increased temperature is known to induce seed dormancy in the related species *Agrostis capillaris (Schonfeld and Chancellor 1983)*. The percentage of GR progeny recovered following SuOP conditions (39.5%) was significantly lower than that recovered from SGT conditions (Table VIII-89). The apparent poor germination of GR seed during the SuOP test relative to the other seed tests may be due to the constant heat stress the young seedlings were exposed to in this study. Heat stress may have weakened seedlings and increased the susceptibility of both the GR and GS plants to the post-SuOP test Roundup treatments. Beard (1973) describes the optimum temperature range for cool season turfgrass growth as 15ºC to 24ºC. 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, some of the GR plants expected to survive following the application of glyphosate may have died due to heat stress, which decreased the observed percentage of GR seedlings and thus the apparent germination success of GR seed. GS seedlings that were expected to die following treatment with glyphosate may have died from both heat stress and the glyphosate treatment. The nearly statistically significant higher percentage of GR seedlings among high germination

energy plants (47.1%) than among low germination energy plants (32.5%) suggests the increased overall mortality from heat stress occurred preferentially among the weak seedlings that germinated later (Table VIII-90). Therefore, seed derived from ASR368 CBG are no more likely to germinate or be more competitive under supra-optimal temperatures than seed that does not possess the glyphosate resistance trait. APHIS is unable to draw a conclusion regarding whether seed derived from ASR368 CBG have greater dormancy under supra-optimal temperatures.

- Accelerated Aging Test (AAT): The AAT can be used as an indication of relative seed survivability or longevity in the soil (Delouche and Baskin 1973), and thus can be used to indicate whether GR CBG seed may be more likely to persist than GS seed. 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**.** Following AAT, seed were tested for viability under standard AOSA temperature conditions for 28 days. The stress conditions of the accelerated aging reduced the growth rate of seedlings throughout the test (Table VIII-92). However, by the end of the test period, SR 1020 seeds had a statistically significant lower germination than ASR368 CBG R1 seeds GT (Tables VIII-88). The total percentage of germinated seedlings from ASR368 CBG R1 seed that were GR was the same following exposure to AAT conditions as under SGT conditions, as were the percentages of GR seedlings with high or low germination energy and seedling vigor (Tables VIII-89 and VIII-90). These results suggest that longevity of ASR368 CBG R1 seed, whether GR or GS, is greater than that of the SR1020 comparator, but that the GR seed showed no greater longevity than the GS seed.

- Additional seed dormancy testing: Hancock and Mallory-Smith (2004) compared the seed dormancy of three GR CBG lines, including ASR368 CBG, to the seed dormancy of SR1020 CBG by suspending seeds in soil at three different depths in two different locations and testing their germination rate every six months. At both sites, seeds of two of the GR CBG lines deteriorated faster than either SR1020 or ASR368 CBG, but the authors speculate this may have been due to difference in seed cleaning procedures. ASR368 CBG seed showed no difference in deterioration from SR1020 CBG seed at one test site, but deteriorated significantly more slowly at the other site, having an estimated half-life of 37.7 months versus 22.8 months for SR1020 CBG seed. This site-specific increase in seed dormancy of ASR368 CBG seed appeared to be due to line by environment interaction and not the *epsps* gene, because the ratio of GR to GS seedlings in surviving ASR368 CBG seed remained 1:1 at all-time points. Although a potential maternal effect of the glyphosate resistance trait on the dormancy of both glyphosate resistant and glyphosate sensitive seed produced by ASR368 CBG plants can't be strictly ruled out (Roach and Wulff 1989; Donohue 2009), there is no obvious mechanism by which the trait would cause such a maternal effect.

- In summary, the data from these diverse germination studies support the conclusion that seed and seedlings of ASR368 CBG are unlikely to demonstrate greater survival, longevity, dormancy or vigor in diverse environments than conventional bentgrass.

The data reviewed above indicate that ASR368 CBG and glyphosate resistant progeny generated by cross-pollination with non-cultivated CBG present in the environment are unlikely to have any greater ability to establish, persist, and spread than non-glyphosate resistant CBG in the absence of glyphosate application. Although most of the data comes from experiments conducted in highly managed environments, the data is indicative of how GRCBG is likely to behave in other environments. Therefore, APHIS concludes that the weed potential (prevalence, competitiveness, damage) of ASR368 CBG or GRCBG derived from it is unlikely to be greater than that of non-glyphosate resistant CBG in managed environments, and with less but still high certainty, in less- and un-managed environments.

However, ASR368 CBG has escaped cultivation in both Oregon and Idaho via seed and possibly pollen, and it's clear that, like non-glyphosate resistant CBG, ASR368 CBG and probably its progeny are able to establish and persist in both highly managed and less managed environments (Watrud *et al*. 2004; Reichman *et al*. 2006; Zapiola *et al*. 2008; Bollman *et al*. 2012; Zapiola and Mallory-Smith 2012; Scotts/Monsanto 2015a, 2015b). Escaped ASR386 CBG plants have established primarily in irrigation and drainage ditches and canals, where they also appear to have spread, and also in artificial ponds, road ditches, landscaping, equipment and waste areas near fields, and newly planted and mature Kentucky bluegrass seed production fields (Reichman *et al*. 2006; Zapiola *et al*. 2008; Bollman *et al*. 2012; Scotts/Monsanto 2015a, 2015b; Mallory-Smith 2016). Dedicated control efforts over the last 12 years appear to have lowered the prevalence of GRCBG (which could include both ASR368 CBG and progeny from hybridization with resident non-cultivated CBG), but GRCBG plants continue to persist in the environment, and may do so for the foreseeable future (Scotts/Monsanto 2015a, 2015b). For instance, in 2013, GRCBG plants were found in a newly planted field of Kentucky bluegrass in Jefferson County (Scotts/Monsanto 2015a). On May 5, 2016, Malheur County Oregon listed GRCBG as a Class A noxious weed which requires that the grass be removed or controlled when found (Meyer 2016).

Since GRCBG can persist in the environment without human assistance, APHIS assessed whether the engineered trait could render GRCBG more difficult to control in situations where CBG is managed as a weed.

Weed Status and Control

A major herbicide used to eliminate CBG where it is not desired is glyphosate. For example, glyphosate is the best control option for management of CBG in other types of

turf and is the most commonly used herbicide to control bentgrasses in grass seed crops, hayfields and pastures, landscapes including home lawns, and US National Parks (Banks *et al*. 2004; Kansas City University 2016). Glyphosate is a preferred herbicide for use in natural areas, public lands, and rights-of-way, both for spot treatments and occasionally for total vegetation control and site preparation prior to renovation with desired species (Banks *et al*. 2004). Glyphosate is the most commonly used herbicide in riparian areas due to its effectiveness, low toxicity, and rapid inactivation (Bollman *et al*. 2012). However, other herbicide options are available for CBG control (Banks et al. 2004; Scotts/Monsanto 2015a, 2015b; Hulting 2016b). Examples include glufosinate, diuron, imazapyr, and the ACCase inhibitors clethodim, sethoxydim, and fluazifop-p-butyl. These vary in number and strength depending on the specific situation.

Riparian Habitats

There is no indication that CBG or GRCBG poses a substantial weed risk to riparian habitats (Banks *et al*. 2004). However, riparian habitats can serve as a source for the spread of GRCBG to other habitats, such as grass seed production fields.

The use of herbicides near standing water is frequently restricted, and glyphosate is the most commonly used herbicide in riparian areas due to its effectiveness, low toxicity, and rapid inactivation (Bollman *et al*. 2012). Other herbicides that could be used against GRCBG in irrigation ditches and other riparian areas are sethoxydim, glufosinate, fluridone, diquat, endothall, and imazapyr (Scotts/Monsanto 2015b). Most of these herbicides are not as effective as glyphosate and/or have other limitations. Sethoxydim is nearly as effective as glyphosate, while glufosinate provides relatively good, though less effective control (control is generally better in young plants) (Reicher and Weisenberger 2002; Butler *et al*. 2002a; Mueller-Warrant 2003; Banks *et al*. 2004; Hancock 2004; Dant and Christians 2005; Hart *et al*. 2005; Butler 2014; Felix 2014; Sbatella and Twelker 2014). However, neither are approved for use in or near surface water sources including irrigation ditches and canals. FIFRA Sec. 24(c) Special Local Need labels have been issued for their use to control GRCBG along dry irrigation canals and drainage ditches in Jefferson and Malheur Counties, Oregon (US-EPA 2016a, 2016b). These labels, which currently expire at the end of 2017, restrict the use of sethoxydim and glufosinate to periods when canals and ditches are dry or contain localized pools of water that will not be used for at least 21 or 14 days after herbicide application, respectively. These restrictions effectively limit use of these herbicides to early spring and late fall (Butler 2014) and may reduce their effectiveness as control measures since they work best on actively growing plants but are usable primarily after CBG has gone to seed or when it likely to be more dormant (US-EPA 2008a; Bollman *et al*. 2012; Butler 2014; Felix 2014; US-EPA 2015a). Soybean oil (Ortho Elementals), propane burning, or other herbicides could be used during the summer to suppress seed production in CBG that survived spring herbicide treatments (Felix 2014; Sbatella and Twelker 2014). Fluridone, diquat, and endothall can all be used in aquatic environments (US-EPA 1995, 2004, 2005), but they are substantially less effective herbicides. Fluridone provides some control of CBG, and diquat and endothall provide poor or no control alone, although

endothall does enhance the level of control provided by fluridone when the two are combined (Butler 2014; Felix 2014). Finally, imazapyr (US-EPA 2006) appears to control CBG as well as glyphosate (Askew *et al*. 2005; Dant and Christians 2005). However, sethoxydim and glufosinate appear to be more widely used to control GRCBG in riparian habitats in Oregon, perhaps because imazapyr has longer soil residual activity (Banks *et al*. 2004). Despite the limitations of these herbicide alternatives to glyphosate, (Scotts/Monsanto 2015a, 2015b) reports success in using them to control non-cultivated GRCBG in Oregon and Idaho. Scotts is currently undertaking research to identify additional herbicides that can be used in and around irrigation ditches while they are in aquatic and semi-aquatic use (Scotts/USDA MOA 2015). APHIS concludes that there are alternatives to glyphosate for the control of GRCBG in riparian habitats including irrigation and drainage ditches and canals, but that limitations on the effectiveness or use of these herbicides renders GRCBG somewhat more difficult to control than glyphosate sensitive CBG in these habitats.

Rights of way and waste areas

As discussed earlier, CBG can grow in rights of way and other disturbed environments with adequate moisture. For instance, some escaped GRCBG plants have been observed in roadside ditches and in waste areas near agricultural fields (Reichman *et al* 2006, Mallory-Smith, personal communication). CBG was not identified in the survey by Banks *et al.* (2004) as a weed problem in these areas, but GRCBG in these areas could be a source for dissemination into other habitats.

Glyphosate is a preferred herbicide for use in rights-of-way environments, where it is used both for spot treatment and total vegetation control. It is particularly useful for site preparation prior to renovation with desirable species because of its lack of residual soil activity (Banks *et al*. 2004). Banks *et al*.(2004) state that imazapic provides good to excellent control of CBG in these settings, though CBG is not currently listed on labels as a targeted species (US-EPA 2008a, 2008b, 2010a; Prather 2016a). Diuron, paraquat, and imazapyr are all used in roadside weed management in the Pacific Northwest, though each has limitations (Prather 2016a). Diuron is generally effective against CBG seedlings but less so against mature plants (Butler *et al*. 2004, 2005a). Paraquat generally kills top growth only and is most effective against seedlings, thus necessitating regular reapplication (Prather 2016a). Imazapyr was discussed above; there is little data on its long term efficacy for CBG control. The most effective alternative to glyphosate is probably the combination of diuron plus bromacil, which has a long lasting residual soil activity (Banks et al. 2004; Hulting 2016c), thus limiting its use for site renovation. However, this should not be a problem if GRCBG occurs as isolated plants or small patches in roadsides and waste areas, since spot treatment should suffice. Indeed, for small patches and roadsides, physical removal should suffice to eliminate and GRCBG plants that occur. The greater challenge is ensuring that all GRCBG plants have been located. APHIS concludes that GRCBG is unlikely to be more difficult to control than glyphosate sensitive CBG in rights of way and waste areas.

Grass seed production fields

Grass seed production in the U.S. is concentrated in the Pacific northwest, particularly Oregon (in 20012, 95% of bentgrass acreage in the U.S., 94% of orchardgrass acreage, 93% of ryegrass acreage, 61% of fescue acreage, and 20% of Kentucky bluegrass acreage) (USDA-NASS 2016a).

Weeds can pose a major challenge in grass seed production, and weed control is especially important during stand establishment in new grass seed production fields (Horton et al. 1990; Holman and Thill 2005). Volunteers and off-types of the same species are the most difficult to control weeds, but other grasses can also be difficult (Chastain 2003a; Affeldt and Butler 2007). Grass seed production utilizes many specialized agronomic practices for weed control, such as carbon-band planting, postharvest residue management (field burning, baling, straw chopping), nutrient and irrigation management to maintain a vigorous crop, early fall application of pre-emergence herbicides to established stands, and mid-fall through early spring application of marginally selective herbicide treatments for controlling young seedling weeds (these herbicide treatments may damage but not kill established perennial plants). Tillage is not widely practiced (except perhaps during seedbed preparation, see below) but remains an option (Chastain 2003a, 2003b; Affeldt and Butler 2007). Seed purity issues, including the presence of other varieties of the same species in the grass crop and the presence of other crops and weeds, often outweigh concerns over yield loss due to weed competition or damage from herbicides (Mueller-Warrant *et al*. 2008).

Where CBG and other *Agrostis* species have historically been grown for seed, they can occur as moderately important weeds in other grass seed crops (Banks *et al*. 2004). Glyphosate is the most commonly used herbicide to control bentgrasses and other grass weeds in grass seed crops. It is used as a spot treatment against bentgrass present in an existing grass seed crop and as a broadcast treatment to remove old grass stands and/or prepare seedbeds prior to planting a new grass seed crop (Mueller-Warrant 2003; Banks et al. 2004; Peachey 2016). In order to assess whether glyphosate resistance renders CBG more difficult to control in grass seed production, APHIS compared the effectiveness and limitations of glyphosate and alternative herbicides within the context of current uses of glyphosate for CBG control.

For seedbed preparation, glyphosate is typically used to remove actively growing weeds. It provides "good" control of bentgrasses (*Agrostis* spp.) in perennial ryegrass, annual ryegrass, tall fescue, fine fescue, bentgrass, and orchardgrass (Peachey 2016), and likely in Kentucky bluegrass as well. However, well-established CBG plants are moderately difficult to control; glyphosate provides good initial kill of top growth, but often allows regrowth from surviving stolon nodes within several months (Mueller-Warrant 2003; Banks *et al*. 2004; Affeldt and Butler 2007; Mueller-Warrant *et al*. 2008). Thus, grass seed producers often follow glyphosate use during seedbed preparation with tillage (Mueller-Warrant 2003). Alternatively, paraquat can be used to provide "good" control of bentgrass seedlings in these grasses (Peachey 2016) and moderate control of somewhat more mature plants (Mueller-Warrant 2003). However, other methods would likely be needed to eliminate mature plants during seedbed preparation, such as mechanical

methods used prior to paraquat application (Peachey 2016). In addition, paraquat is highly toxic and is a "restricted-use pesticide" that only certified applicators are authorized to apply (US-EPA 1997a, 1997b, 2015a). Another alternative is to use glyphosate or other herbicides to remove most plants followed by mechanical methods such as double disking to remove any plants that survive herbicide treatment (Butler *et al*. 2005b). Other herbicides are used during planting or stand establishment of various grass seed crops to eliminate weed seedlings, including the soil active long-residual herbicides diuron and pronamide, which are used in activated carbon band planting, and other herbicides including, ethofumesate, glufosinate, and mesotrione (Rinehold and Jenkins 1994; US-EPA 2002, 2003, 2008b, 2010b, 2012, 2013a; Scotts/Monsanto 2015a; US-EPA 2015b; Peachey 2016). Most importantly, glyphosate is not used during planting or stand establishment of grass seed crops. Therefore, glyphosate resistance would not be expected to alter management practices for control of CBG seedlings during planting and stand establishment.

Glyphosate is also used for spot treatment of CBG growing in established grass seed production fields and is the single most effective control option currently registered for this purpose in most grasses. However, as previously noted, well-established CBG plants are moderately difficult to control even with glyphosate. Thus, multiple glyphosate treatments are often required for complete control and even with multiple treatments complete control may not be achieved (Mueller-Warrant 2003; Banks *et al*. 2004). Rather, a minimal standard for success is preventing the treated plants from going to seed during the production season of the desired crop (Mueller-Warrant 2003). A number of other herbicides could be used to control GRCBG in established grass seed productions fields. However, they have various limitations (Table 1, page 31), and there is a lot of variability in their efficacy depending on environmental conditions (Hulting 2016b). Many of these herbicides are only effective on CBG seedlings (Peachey 2016), which may be difficult to detect in mature stands of other grasses. There is a much more limited selection of herbicides available for use against mature GRCBG plants in other grass seed crops. Although glufosinate could be used against mature plants, as in riparian settings, it is much less effective than glyphosate because it is a contact herbicide that has limited translocation through the plant (Vencill 2002; Butler *et al*. 2002a; Mueller-Warrant 2003; Askew *et al*. 2005; Hart *et al*. 2005). Thus, effective control with glufosinate requires good coverage with extensive spraying for effective control, which could result in severe injury to grass seed crops of some species; in contrast, glyphosate can be applied in a more targeted fashion with a roller, wick or wiper (Peachey 2016). Fluazifop-P and sethoxydim can approach or exceed the efficacy of glyphosate (Reicher and Weisenberger 2002; Butler *et al*. 2002a; Mueller-Warrant 2003; Hancock 2004; Hart *et al*. 2005), but their use is currently restricted to fine fescue production fields (Peachey 2016) and they may provide inconsistent control, possibly dependent on location and season of application (Hart *et al*. 2005). Terbacil and various diuron and terbacil combinations appear to have good efficacy against mature CBG and/or mature GRCBG in Kentucky bluegrass (Butler *et al*. 2004, 2005a). When used at higher doses and/or two or more applications, mesotrione provides fair to good control of CBG in perennial ryegrass, tall fescue, and Kentucky bluegrass (Askew *et al*. 2005; Branham *et al*. 2005; Butler *et al*. 2005a; Beam *et al*. 2006; Affeldt and Butler 2007; Jones and Christians

2007; Kaminski and Machnicki 2008; Dernoeden *et al*. 2008a). However, no more than two applications per year are permitted (Peachey 2016). Kentucky bluegrass growers also use hand hoeing and hand pulling to remove weeds (Chastain 2003a). Most of these herbicides also have restrictions on use near surface water and thus could be used only away from irrigation channels. There do not appear to be alternative herbicides for control of mature GRCBG plants in annual ryegrass, orchardgrass, or creeping bentgrass fields.

Table 1. Effectiveness of Herbicides for Spot Treatment of GRCBG in Grass Seed Production Fields (Peachey 2016)

	Grass Seed in Production						
	Annual Ryegrass	**Perennial Ryegrass**	**Tall Fescue**	**Fine Fescues**	**Orchard grass**	**Kentucky Bluegrass[1]**	**Bentgrass**
Effective against seedlings and mature CBG							
Glyphosate[2]	Good	Good	Good	Good	Good	Good	Good
Ethofumesate[3]			Good?				
Fluazifop-P[4]				Fair-Good			
Mesotrione[1]		Fair-Good	Fair-Good			Fair-Good	
Sethoxydim[5]				Fair-Good			
Terbacil[6]						Good	
Diuron, Terbacil combinations[6,7]						Good	
Effective against CBG seedlings only							
Dimethenamid-P[6]		Good	Good	Fair	Good	Good	Fair-Good
Diuron[8]		Good	Good	Good	Good	Good	Poor-Good
Ethofumesate[3]	Good	Good	Good			Good	Fair
Glufosinate[9]	Fair	Fair	Fair	Fair	Fair	Fair	
Mesotrione	Not rated						
S-Metolachlor[6,10]		Good	Good	Fair	Good	Good	Fair-Good
Metribuzin[11]		Good	Good	Good	Good	Good	Poor-Good
Oxyfluorofen[6,12]		Good	Good	Good	Good	Good	Poor-Fair
Pendimethalin		Good	Good	Good	Good	Good	Fair-Good
Primisulfuron-methyl[6]						Not rated	
Pronamide[13]		Good	Good		Good		
Terbacil[6]			Not rated	Not rated			
Flufenacet+ metribuzin[14]		Good	Good	Good	Good		

[1] Effectiveness rating based on comparison to ratings in other grass seed crops, except rating for control of mature CBG by mesotrione based on (Askew *et al*. 2005; Branham *et al*. 2005; Butler *et al*. 2005a; Beam *et al*. 2006; Affeldt and Butler 2007; Jones and Christians 2007; Kaminski and Machnicki 2008; Dernoeden *et al*. 2008a) and ratings for control of mature CBG by diuron and terbacil combinations and by terbacil based on (Butler *et al*. 2004, 2005a).

[2] Multiple applications may be required for complete control of mature CBG

[3] Reported studies suggest ethofumesate provides poor control of both seedling and mature CBG (Meyer and Branham 2006; Kaminski and Machnicki 2008; Dernoeden *et al*. 2008b), some CBG cultivars have good to excellent tolerance (US-EPA 2012, 2013a), and the label of Nortron indicates that it is used in mature CBG to control other grasses (US-EPA 2015b). However, Hulting (personal communication)

(Hulting 2016b) reports that it provides fair to good control of grass weeds in most grass seed production field, with efficacy highly dependent on environmental conditions. Use in ryegrass is permitted only in Western Oregon; after treatment, can't replant to other than sugar beet or ryegrass for one year;
[4] Apply to actively growing grasses 2- 4 inches tall; slower to act than but nearly equally as effective as glyphosate; generally requires higher rate or second application for adequate control of mature plants (Reicher and Weisenberger 2002; Mueller-Warrant 2003; Hart *et al.* 2005)
[5] Slower to act than but approaches effectiveness of glyphosate; generally requires multiple treatments for adequate control of mature plants and complete control is rare (Banks, 2004 #17;Felix, 2014 #445;Butler, 2014 #444;Butler, 2002b #404;Hancock, 2004 #419;Hart, 2005 #96;(Reicher, 2002 #170;Sbatella, 2014 #368)
[6] Applying while grass crop is under stress may cause crop injury
[7] Combinations of diuron with oxyfluorfen and terbacil, and of terbacil with primisulfuron-methyl
[8] Injures Kentucky bluegrass and orchardgrass if applied at the maximum rate for two consecutive years
[9] Grower assumes all liability for crop injury
[10] Apply only once per crop season
[11] Apply when weeds are in 1-2 leaf stage
[12] Apply before weed seedlings exceed 2 leaf stage; application when crop plants have less than one tiller may result in severe injury or stand loss
[13] Restricted-use herbicide
[14] Apply before weed seedlings exceed 2 leaf stage; may cause crop injury depending on when applied and other herbicides used; not permitted in Jefferson County, OR

Even if GRCBG were not completely controlled in grass seed production fields, it would not be expected to have any greater negative impact on grass seed purity than glyphosate sensitive CBG. First, it would be unlikely to produce viable seed due to differences in maturity timing and harvest schedules. For example, in the primary grass seed production area in Oregon (Willamette Valley Wineries Association 2016), cultivated CBG is typically harvested in late July or August, while annual and perennial ryes, and tall and fine fescues are harvested two to five weeks earlier, when most CBG seed would still be immature (Bush *et al*. 2000; Scotts/Monsanto 2015b; Stamm *et al*. n.d.). In Jefferson County, Oregon, Kentucky bluegrass is typically swathed in early July and takes 7 – 10 days to dry before it is combined (Butler *et al*. 2002b ; Scotts 2013), while cultivated CBG is typically swathed in early August (Scotts 2013). Thus, CBG seed is unlikely to be viable even if present as an impurity. Second, CBG seed is approximately 1/25 to 1/30 the size of perennial rye and tall fescue seed, 1/10 to 1/15 the size of fine fescue and orchardgrass seed, and 1/3.5 to 1/6 the size of Kentucky bluegrass seed (Bush *et al*. 2000; Stahnke *et al*. 2010). Thus, most CBG seed that might intermix with seed of these other species is removed in combines and various seed cleaners in downstream conditioning systems. Indeed, after the dissemination of ASR368 CBG from experimental fields into Kentucky bluegrass fields in 2003, many ASR 368 CBG plants remained after rogueing and herbicide applications were employed to mitigate the volunteers. Thus, for four years all seed lots from fields containing ASR368 CBG plants were quarantined and evaluated for the presence of ASR368 CBG seeds. No seeds were identified in a total of 102 lots tested (Table VIII-113, Scotts/Monsanto 2015a).

In summary, alternatives to glyphosate for control of CBG during grass seed bed preparation or in mature grass seed fields are limited. For seedbed preparation, paraquat is not as effective as glyphosate and is more toxic, and other herbicides are less effective still, necessitating more labor intensive control efforts. For spot treatment of mature CBG in grass seed production fields there are no herbicide alternatives in annual ryegrass,

orchardgrass, or creeping bentgrass. Mesotrione could be used in perennial ryegrass, tall fescue, and Kentucky bluegrass, and additional alternatives are available for fine fescues and Kentucky bluegrass, but with some limitations on their use. Currently, GRCBG occurs in regions where Kentucky bluegrass, fine fescue, and perennial ryegrass are grown for seed production. Alternatives to glyphosate are available for all of these grass seed crops, although they are limited to mesotrione for perennial ryegrass. APHIS concludes that, depending on the grass being cultivated for seed, control of GRCBG is likely to be somewhat to substantially more difficult in grass seed production fields than control of glyphosate sensitive CBG. However, even if some GRCBG plants remain, seed from the plants is extremely unlikely to be present as an impurity in other grass seed due to differences in seed maturity timing and harvest schedules and to seed cleaning procedures.

Turf and landscaping

CBG is a moderately important weed in golf turf (Banks *et al*. 2004). Although it can occur in other turf and landscapes, such as commercial and residential lawns and recreation areas, it is not generally considered a problem in these locations (Banks *et al*. 2004). However, these locations could provide a source for spread of CBG to other areas (Ahrens *et al*. 2011a). Glyphosate is the best control option currently available for management of CBG in other types of turf, and the standard recommendation is to spot treat and then reseed or re-sod the treated area (Banks *et al*. 2004). However, even with glyphosate effective control of CBG in established turf is difficult, for two reasons. First, it's difficult to see recently established stolons and small bentgrass patches in dense turf and thus it's unlikely that all bentgrass will be observed and treated. Second, where treatment is attempted, a small percentage of stolons or stolon sections often survive, allowing the grass to reestablish. Multiple applications of glyphosate are generally required (Banks *et al*. 2004; Beam *et al*. 2006; Jones and Christians 2007). Thus, CBG control in other turfgrasses is not commonly attempted, and most homeowners and professional turf managers generally either keep the CBG infested turf or destroy the entire turf and reseed or resod (Banks *et al*. 2004). However, should control be attempted, foramsulfuron provides good to excellent control in the major warm season turfgrasses: bermudagrass, zoysiagrass, and buffalograss (Banks *et al*. 2004; US-EPA 2015e). Mesotrione, discussed in the previous section, provides fair to good control in the major cool season turfgrasses (other than creeping bentgrass itself): Kentucky bluegrass, perennial ryegrass, and tall fescue (US-EPA 2010b). Therefore, APHIS concludes that GRCBG is unlikely to be more difficult to control than glyphosate sensitive CBG in turf.

Hayfields and pastures

Hayfields may be alfalfa or grass, while productive pastures generally combine a grass crop and a legume. Typical grasses include orchardgrass, tall fescue, annual and perennial ryegrass, and timothy grass, while alfalfa and clovers are typical legumes (Fransen and Chaney 2002; Wilson and Orloff 2006).

CBG and other *Agrostis* species sometimes occur in hayfields and pasture (usually in riparian areas) but are considered to be of low importance since they are utilized by livestock without deleterious effects and few efforts are made to control or manage them (Banks *et al*. 2004). Indeed, CBG is sometimes used as a forage species in exercise or confinement areas (Fransen and Chaney 2002). The finding by (Banks *et al*. 2004) that few efforts are made to control or manage *Agrostis* species in hayfields and pasture is consistent with general recommendations that herbicides and tillage not be used in pastures until weeds have become so dense that the site is unprofitable and must be renovated (Hulting 2016c). Instead, pasture is managed to prevent or reduce weed problems by selecting appropriate forage species, grazing management, and appropriate fertilization and water management to improve the competitiveness of desirable forage species. Spot treatment with herbicides can be useful for controlling weeds that are new and few in number, and hand weeding can be very effective though time consuming and tedious (Wilson and Orloff 2006; Whitesides and Bouck 2010; Hulting 2016c).

According to Banks *et al.* (2004), glyphosate is occasionally used for spot treatment of weeds and for renovation of hayfields and pastures, and it provides excellent control of CBG in pasture and rangeland. It is the recommended herbicide for use in hayfield and pasture renovation (Fransen and Chaney 2002; Hulting 2016c). However, since glyphosate is not generally used in hayfields or pastures there would typically be no selective pressure favoring GRCBG and it would generally not be more prevalent as a weed than glyphosate sensitive CBG. An exception may be fields of glyphosate resistant alfalfa (USDA-APHIS-BRS 2010), where any GRCBG present would survive glyphosate applications. Several additional herbicides effective against CBG are available for use as spot treatments in alfalfa hayfields. These include diuron, metribuzin, paraquat, pendimethalin, pronamide, sethoxydim, and terbacil (Prather 2016b). The strengths and weaknesses of these herbicides are discussed above. Among these, sethoxydim is effective against mature CBG plants. The related herbicide clethodim can also be used in alfalfa hayfields and is comparably effective against mature CBG plants (Reicher and Weisenberger 2002; Mueller-Warrant 2003; Hancock 2004; Hart *et al*. 2005; Butler *et al*. 2005a; Butler *et al*. 2005b). Both sethoxydim and clethodim are most effective on actively growing grasses (Prather 2016b). In the highly unlikely event that GRCBG plants became very prevalent in alfalfa hayfields requiring renovation, growers would likely need to use alternative methods for renovation, including discing or planting a cleanup crop followed by discing before replanting to alfalfa (Fransen and Chaney 2002). Double discing provides adequate control for eliminating a seed field of creeping bentgrass even in the absence of herbicide (Butler *et al*. 2005a; Butler *et al*. 2005b), and should be similarly effective for alfalfa hayfield renovations.

For control of CBG in grass hayfields, and in hayfields and pastures of mixed grasses and alfalfa there are many fewer alternatives. Indeed, weed control in general is more difficult in mixed alfalfa/grass stands (Wilson and Orloff 2006). Dimethenamid-P can be used in grass hays and flufenacet + metribuzin can be used specifically on Timothy grass hay (Prather 2016b, labels). Pendimethalin, paraquat, and imazapic can be used in all three situations (US-EPA 2004, 2008b, 2010a; New Mexico State University 2013; US-EPA 2015b; Prather 2016b; Hulting 2016c; US-EPA 2016d). In addition, metribuzin could be

used in grass and mixed grass/alfalfa hays (Wilson and Orloff 2006) while imazapyr could be used in grass hay and grass pasture (New Mexico State University 2013). With the exception of imazapyr, none of these herbicides appear to be very effective against mature CBG plants (Elmore *et al*. 2015) (and references cited above).

APHIS concludes that GRCBG is unlikely to pose a substantial weed problem in hayfields, pasture, or rangeland and that efforts to control GRCBG are unlikely to be undertaken. If control of GRCBG is desired, it may be more difficult to control than glyphosate sensitive CBG in grass hayfields and mixed grass/alfalfa hayfields and pastures. It will generally not be more difficult to control in alfalfa hayfields except in the extremely unlikely situation that field renovation is required and GRCBG is highly prevalent in the field.

Fruit crops

CBG is reported to pose a problem of low importance in some fruit crops in Oregon. Glyphosate and glufosinate are the primary herbicides used to control CBG in fruit crops (Banks *et al*. 2004). Glufosinate provides an alternative means of control in these crops that works about as well as glyphosate, and many other herbicides are also available (Banks *et al*. 2004). Therefore, APHIS concludes that GRCBG is unlikely to be more difficult to control than glyphosate sensitive CBG in fruit crops.

Conclusion - Conventional CBG vs Glyphosate-resistant (ASR368) CBG

Based on the agronomic field data and literature survey reviewed above, ASR368 CBG demonstrates no consistent and substantive differences from conventional bentgrass in seedling vigor, establishment ability, growth rate, vegetative vigor and competitiveness, survival, flowering and pollen characteristics, or fecundity, and very likely no substantive differenced in seed germination and dormancy, . The *epsps* transgene conferred no fitness advantage to ASR368 CBG in the absence of glyphosate application. Although no experiments were conducted with wild/feral CBG which acquired the *epsps* gene from ASR368 CBG, the lack of substantive differences between ASR368 CBG and other cultivars strongly suggests there will similarly be no fitness differences between wild/feral CBG with or without the transgene in the absence of glyphosate application. Thus, glyphosate resistant creeping bentgrass is unlikely to be more competitive, or become more prevalent or damaging, than glyphosate sensitive creeping bentgrass in the absence of glyphosate application.

Scotts/Monsanto has no intention to commercialize or maintain seed stocks of ASR368 CBG and is actively undertaking eradication efforts to remove GRCBG from the environment in Oregon and Idaho. GRCBG incidence in the environment is likely to continue decreasing until it reaches a very low level as long as eradication programs continue.

ASR368 CBG differs from conventional CBG mainly in its ability to tolerate exposure to the herbicide glyphosate. The elimination of glyphosate as a useful herbicide for GRCBG, and the limitations of other herbicides, render GRCBG somewhat more difficult to control than glyphosate sensitive CBG in riparian areas, grass seed production fields, and some hayfields and pastures. However, ASR368 CBG and feral GRCBG can nonetheless be managed using a variety of currently available methods, including mechanical and cultural methods and alternative herbicides (Scotts/Monsanto 2015a). Therefore, APHIS concludes that GRCBG is unlikely to pose a significant weed problem and that any adverse consequences from the escape and persistence of GRCBG in the environment are unlikely.

G. Potential Impacts on the Weediness of Any Other Plants with which ASR368 Creeping Bentgrass Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Soltis *et al*. 1993; Reisberg 1997; Hegde *et al*. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Reisberg 1997). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury *et al*. 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (Table 1, Ellstrand *et al*. 1999). This topic is covered in two aspects: 1) the potential for gene flow, hybridization and introgression from ASR368 CBG or feral GRCBG containing the *cp4 epsps* gene to sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories, and 2) if so, the risk potential with respect to weediness of those related taxa based on the phenotypic changes that have been observed in the engineered plants.

Potential for gene flow, hybridization and gene introgression

Hybridization and gene introgression from one plant species to another are multi-step processes that begin with cross pollination between plants of the different species, followed by the production of fertile hybrid seed, the persistence of hybrid plants through reproductive maturity for one or more generations, and in the case of gene introgression, recurrent backcrossing of hybrids to plants of the parent species lacking the gene until the gene is incorporated into the genome of (a population of) that species (Stebbins 1958).

CBG can form hybrids with many *Agrostis* (bentgrass) and *Polypogon* (rabbitsfoot grass) species found in the contiguous United States (Table 2). Natural (i.e., non-experimental) hybrids of CBG have been described with all of these species except *A. pallens* and *A. idahoensis*, although there is some question about whether natural hybrids with *A. exarata* and *A. scabra* were formed (Table 2, MacBryde 2006). Some of these species can also readily hybridize with each other. For instance, *A. capillaris* can readily hybridize with both *A. casteallana* and *A. gigantea* (MacBryde 2006). Some of the interspecific hybrids are common enough in nature that they have been given scientific

names (e.g., hybrids of CBG and *A. capillaris* have been named *A. x murbeckii*, while hybrids of CBG and *P. monspeliensis* are x *Aprogon lutosis* (syn. x *Agropogon littoralis*) (MacBryde 2006). Natural hybrids between CBG and additional species of *Agrostis* have been found elsewhere in the world, but these other species are not found in the contiguous U.S. (MacBryde 2006). No hybridization data have been reported for other *Agrostis* species found in the contiguous U.S. (MacBryde 2006); this lack of data is a source of uncertainty. None of the species with which *A. stolonifera* can hybridize are listed as threatened and endangered in the U.S. and none are on the Federal noxious weed list.

Table 2. Species of *Agrostis* and *Polypogon* Currently in the Contiguous United States (including Idaho and/or Oregon) Which can Hybridize With *A. stolonifera*

Species and common name	Status in Contiguous U.S.[1]	Range in Contiguous U.S.[2, 3]	Weed status in Contiguous U.S.[4, 5]	Ploidy Level or Genome Constitution Similar to CBG[1, 6]	Hybridization with CBG[1, 7, 8, 9, 10] (Frequency and hybrid fertility)
***Agrostis canina* Linnaeus** (Velvet bentgrass)	Naturalized, Agronomic Use	OR, MI to ME to TN	Low importance in turfgrass	No	Rare Very low fertility
***Agrostis capillaris* Linnaeus** (Colonial bentgrass)	Naturalized, Agronomic Use	Most Eastern & Western states but few midcontinent	Low to moderate importance; greatest concern in grass seed fields	Yes	Occasional Variable fertility (sterile to moderate fertility)
***Agrostis castellana* Boissier & Reuter** (Dryland bentgrass)	(Naturalized), Agronomic Use	CA, OR, WA	Very low importance in turfgrass	Yes	Infrequent Very Low to Moderate fertility[12]
***Agrostis exarata* Trinius** (Spike redtop or bentgrass)	Native	Western US, KY, VT	Low to moderate importance; greatest concern in grass seed fields	Ploidy - Yes Genome - unknown	Uncertain (suspected in OR, WA)[11] Sterile
***Agrostis gigantea* Roth** (Redtop)	Naturalized, Agronomic Use	All states	Low to moderate importance; greatest concern in grass seed fields	Ploidy – No Genome - Yes	Occasional Variable fertility (sterile to moderate fertility)

***Agrostis idahoensis* Nash** (Idaho redtop or bentgrass)	Native, Minimal Agronomic use	Western US	Low to moderate importance; greatest concern in grass seed fields	Ploidy – Yes Genome - Unknown	Experimental only Nearly sterile
***Agrostis mertensii* Trinius** (Northern or Arctic bentgrass)	Native	ME to NY, WV to SC, TN, CO, MO, UT, WA, WY	No reports found	Ploidy – No Genome - Unknown	Rare Fertility not reported
***Agrostis pallens* Trinius** (Leafy, Dune, or Seashore bentgrass)	Native	CA, ID, MT, NV, OR, UT, WA	No reports found	Ploidy – No Genome - Unknown	Experimental only Fertility not reported
***Agrostis scabra* Willdenow** (Rough bentgrass, Ticklegrass)	Native	All states	Low to moderate importance; greatest concern in grass seed fields	Ploidy - No Genome - Unknown	Experimental only Fertility not reported
***Polypogon monspeliensis* (Linnaeus) Desfontaines** (Annual Rabbitsfoot grass)	Naturalized	All states except IA, IL, IN, KY, OH, MO, VT, WV	Low to moderate importance in vegetable and row crops; weedy[13]; troublesome invader in CA wetlands[14].	Ploidy – Yes Genome - unknown	Infrequent Almost completely sterile, only F1 generation is known[10,15,16,17]
***Polypogon viridis* (Gouan) Breistroffer** (Beardless Rabbitsfoot grass)	Naturalized	Southwestern US	No weed reference found	Yes – ploidy Unknown - genome	Rare; Sterile

[1](MacBryde 2006); [2] PLANTS Database; [3] Kartesz 2010; [4](Banks *et al*. 2004); [5] (CABI 2012); [6] (Honig *et al*. 2015); [7] (Wippf 2002); [8] (Christoffer 2003); [9](Belanger *et al*. 2003a); [10](Zhao *et al*. 2007); [11] (Carlbom 1966) [12]; Moderate female fertility; Moderate male fertility in backcross to CBG, but very poor in backcross to *A. castellana*;[13] ;(USDA-NRCS 2016d) [14] ;(Zedler and Kercher 2004); [15] (Zapiola and Mallory-Smith 2012); [16](Bradshaw 1975); [17] (Hubbard 1984)

Among the species that occur in the contiguous U.S., the interspecific hybrids of CBG that are most commonly observed and most likely to occur in nature are hybrids between CBG and *A. capillaris*, *A. gigantea*, and *P. monspeliensis* (Widén 1971; Banks *et al*. 2004; MacBryde 2006). However, there are few reports of these or other interspecific hybridizations with CBG occurring in the U.S. outside of experimental field studies (Carlbom 1966; Watrud *et al*. 2004; Zapiola and Mallory-Smith 2012). The reason for the apparent rarity of these hybrids in the U.S. is not known, but could include genetic, environmental, and ecological factors affecting the frequency of cross pollination, the

production of fertile hybrid seed, and the fitness and reproductive vigor of hybrid plants (Bradshaw 1958; Widén 1971; Belanger *et al*. 2003b; Banks *et al*. 2004; MacBryde 2006). Alternatively, hybrids could be more common but unrecognized, particularly in patches of grasses, given the relatively similar morphology of the various *Agrostis* species, the variable, intermediate and sometimes unpredictable morphologies of hybrids, and the lack of reports of efforts by specialists to look for them (Bradshaw 1958; Widén 1971; MacBryde 2006; Zapiola and Mallory-Smith 2012; Scotts/Monsanto 2015a).

CBG and most of its sexually compatible relatives are largely wind-pollinated outcrossing perennials, although they can self-pollinate at a low rate (<1%). However, *P. monspeliensis* is a highly self-pollinating annual, and *A. exarata* and *A. scabra*, are both self-pollinating perennials (Carlbom 1966; Widén 1971; Christoffer 2003; Belanger *et al*. 2003b; Banks *et al*. 2004; MacBryde 2006; Zapiola and Mallory-Smith 2012). Like CBG, many of these species are capable of vegetative spread and clonal propagation via stolons and/or rhizomes (Davies 1953; Widén 1971; Dore 1980; Edgar and Forde 1991; MacBryde 2006). There are thus three pathways by which transgene flow and subsequent introgression could occur between ASR368 CBG, or feral GRCBG harboring the *cp4 epsps* transgene, and sexually compatible species: 1) persistence and asexual vegetative spread and propagation of first generation (F1) hybrid plants; 2) recurrent backcrossing of F1 hybrids with the sexually compatible species and persistence of the CBG-derived transgene in multiple subsequent generations; and 3) generation of subsequent hybrid generations through inter-pollination among F1 hybrid plants, forming a "hybrid swarm" of individuals with a gradation of phenotypes between the two parent species, with persistence of the CBG-derived transgene.

The first steps in each of these pathways are pollination between GRCBG and one of the other species followed by the production of viable hybrid seed. Given the outcrossing nature of most of these species, cross-fertilization could occur in either direction, from GRCBG to another species or from the other species to GRCBG. An exception is *P. monspeliensis* which, being highly self-pollinating, is more likely to be the pollen donor than the pollen recipient since any pollen from GRCBG which lands on *P. monspeliensis* stigmas will be in competition with a large amount of *P. monspeliensis* pollen for fertilization. Several factors can influence the level of cross pollination, including 1) the degree of synchrony (date and time of day) of pollen shed and receptive stigmas between species, 2) the spatial arrangement of pollinating and receptor plants (distance, distribution, and population size), 3) wind speed and direction, and 4) pollen viability and longevity in the context of climatic conditions (Burton 1992).

Several studies have found at least partial overlap in both flowering and pollen shed-stigma receptivity between CBG and many of the species listed in Table 1, including substantial overlap between CBG and *A. capillaris* in the United Kingdom, Oregon, and New Jersey (Davies 1953; Bradshaw 1958; Christoffer 2003; Belanger *et al*. 2003a). These and other studies also demonstrate that the order and degree of overlap in flowering date, duration, and time of pollen shed can vary depending on genetic factors (Tables VIII-63 and VIII-64, Scotts/Monsanto 2015a) and environmental factors (Widén 1971; Christoffer 2003), and probably also due to interactions between genetic and

environmental factors. The start date and duration of anthesis of ASR368 CBG were not substantively altered in ASR368 CBG compared to non-transgenic comparator varieties in field trials conducted in Jefferson County, Oregon in 2002 (Tables VIII-63 and VIII-64). Thus, GRCBG appear no more or less likely to form hybrids with other *Agrostis* or *Polypogon* species than non-transgenic CBG due to changes in the timing of flowering and pollen release.

CBG pollen remains viable for of 30 minutes to 3 hours after it is shed (Fei and Nelson 2004), and can travel long distances (up to 12 miles or more) to successfully pollinate receptive plants; however, the rate of cross-pollination decreases rapidly with increasing distance (Wipff and Fricker 2001a; Christoffer 2003; Belanger *et al*. 2003b; Watrud *et al*. 2004; Reichman *et al*. 2006). Although the longest distances were observed from large cultivated fields of CBG, even relatively small groups of CBG plants can act effectively as a pollen source for receptive sexually compatible species up to at least 1000 feet away (Christoffer 2003; Belanger *et al*. 2003b). However, the frequency of cross-pollination is likely to be very low for plants more than 325 feet apart (Christoffer 2003; Belanger *et al*. 2003b).

No differences in pollen size, viability or longevity were observed between ASR368 CBG pollen and pollen from other CBG cultivars, indicating that pollen from GRCBG is unlikely to differ from non-transgenic CBG in its ability to disperse, pollinate, or fertilize other sexually compatible plants (Tables VII-66 to VII-72, Scotts/Monsanto 2015a).

CBG and many of its sexually compatible relatives share similar though non-identical habitats (Widén 1971; Dore 1980; Edgar and Forde 1991; Harvey 2007; Scotts/Monsanto 2015a) which could bring them into close enough proximity to enable cross pollination to occur. In particular, many of these species prefer generally moist, often disturbed, areas. Typical habitats include pastures, hay fields, moist meadows, the banks or edges of lakes, ponds, marshes, rivers, streams, creeks, canals, and ditches, and road sides and waste lands. Wetland and riparian areas (streams, canals, and irrigation ditches) provide the best habitat for CBG and most of the other species present in the same counties as GRCBG (*A. capillaris, A. exarata, A. gigantea, A. idahoensis, A. pallens, A. scabra,* and *P. monspeliensis*) (Watrud *et al*. 2004; Reichman *et al*. 2006; Zapiola *et al*. 2008; Scotts/Monsanto 2015a; Consortium of Pacific Northwest Herbaria 2016). Co-localization of GRCBG, *A. exarata,* and *A. gigantea* plants has been observed in field surveys in Oregon, and irrigation ditches in eastern Oregon are commonly seeded with *A. gigantea* at the waterline to control weeds (Watrud *et al*. 2004; Reichman *et al*. 2006; Scotts/Monsanto 2015b).

Based on the comparison between the biological characteristics of ASR368 CBG and non-engineered CBG presented in Section F above, the *cp4 epsps* transgene is unlikely to change the habitat distribution of GRCBG relative to non-engineered CBG. For example, comparison of seedling establishment in Oregon demonstrated that ASR368 CBG exhibited no increased ability to establish and survive in irrigated conditions compared to four non-transgenic CBG cultivars, and showed only transiently greater ability to establish and survive in non-irrigated conditions than one of the four cultivars in one of two years (Tables VIII-11 and VIII-12, Scotts/Monsanto 2015a). ASR368 CBG also

showed no significant and consistent increase in vegetative establishment from stolons than other CBG cultivars under either irrigated or non-irrigated conditions in Oregon and three other states (Tables VIII-15 to VIII-21). For all cultivars, vegetative establishment under non-irrigated conditions in Oregon was very poor, consistent with the known habitat requirements of CBG.

There is little data available on the viability of F1 hybrid seed resulting from cross pollination between CBG and its sexually compatible relatives. Davies (1953) reported very low numbers of seedlings produced by self-fertilization of CBG, *A. capillaris, A. canina*, or *A. gigantea* (range 0 – 7 per panicle). However, 8 – 32 times more seedlings were produced by cross-pollination of CBG and *A. capillaris* plants (31 – 178 per panicle), while over 275 times more from cross-pollination with *A. gigantea* as the male parent (366 per panicle; but only two per panicle when *A. gigantea* was the female parent). Crosses between CBG and *A. canina* were largely unsuccessful, producing only 1 – 3 seedlings per panicle. In contrast, in more recent experiments, Belanger *et al* (2003a) reported both a low number of plants produced per panicle upon self-fertilization of *A. capillaris, A. canina, A. castellana, and A. gigantea* (0.5 – 1.1 plants/panicle) and an even lower production of hybrid plants upon crossing with CBG, even though the panicles of the CBG and sexually compatible plants were bagged together to facilitate cross-pollination. CBG produced more hybrids with *A. capillaris* and *A. gigantea* (0.2 and 0.9 F1 hybrid plants per panicle) than with the other two species (0.1 F1 hybrid plants per panicle). No data is available concerning the viability of hybrid seed produced in crosses of ASR368 CBG with sexually compatible relatives.

Several recent experiments provide additional indications that the rate of hybrid formation between CBG and other species is low even when the plants are close together. In experiments conducted in New Jersey, Belanger *et al.* (2003b) found that the frequency of hybrid recovery from a central plot of five CBG pollen donors and a surrounding array of *A. capillaris, A. castellana, A. gigantea, A. canina*, and *A. capillaris* planted at 3 meter intervals to a maximum of 15 meters was approximately 0.5% and 0.005% respectively at 3 meters, and 0.044% and 0.0015% respectively across the entire array (44,967 and 663,778 plants seedlings screened respectively). In this experiment, *A. capillaris* and *A. castellana* produced hybrids with CBG at, respectively, $1/10^{th}$ and $1/400^{th}$ the rate at which CBG produced hybrids with other CBG plants (2% at 3 meters and 0.6% overall). No hybrids were formed with *A. gigantea* (out of nearly 2.3 million seedlings screened; no hybrids were formed with *A. canina* either, but only 7556 seedlings were tested). In a study that examined hybrid formation from a large CBG pollen source (>400 cultivated acres spread over an 11,000 acre area), dozens of hybrids were formed with resident (naturalized) CBG and resident *A. gigantea* located within 10 miles of the nearest CBG field, at an overall rate of 0.03% and 0.04% respectively (565,000 and 397,000 seedlings tested), compared to a rate of 2.00% hybrid formation among sentinel cultivated CBG plants (APVMA Approval No 6763256505 nd) (Watrud *et al*. 2004). Most of the hybrids were formed from receptor plants located within 2 miles of the nearest CBG field. However, these data likely greatly overestimate the frequency with which hybrids will form from the small source populations of GRCBG currently present in the environment (Scotts/Monsanto 2015b).

Based on the data and information reviewed above, APHIS concludes that GRCBG is no more likely to form viable hybrid offspring with sexually compatible relatives than non-transgenic CBG, and that the risk of hybridization of GRCBG currently present in the environment with sexually compatible relatives is extremely low.

As discussed above, once hybridization occurs there are three pathways by which transgene flow and introgression from GRCBG to a sexually compatible species could occur. In the first pathway, hybrid offspring persist and spread vegetatively. This pathway will be discussed further in the next section. In the second and third pathways, transgene introgression from GRCBG to a sexually compatible species occurs either by repeated backcrossing to the non-CBG parent with inheritance of the transgene through multiple generations (pathway 2) or by inbreeding among hybrid plants to generate a "hybrid swarm" of individuals with varying levels of parental traits (pathway 3). However, if the hybrid plants are both male and female sterile, there would be no possibility for further gene introgression beyond the F1 stage. Therefore, APHIS examined data on the fertility of F1 hybrids.

Table 2 provides information on similarities in the genome constitution or ploidy level of CBG and its sexually compatible species; similarities would generally favor the ability to produce F1 hybrids capable of sexual reproduction (MacBryde 2006). Table 2 also provides a summary of available data on the level of fertility of F1 hybrids. Most of the available data relates only to male (pollen) fertility, there is little data related to female (ovule) fertility. All F1 hybrids of CBG with other species have substantial decreases in fertility. Some hybrids demonstrate complete sterility others appear to have a variable degree of fertility. In particular, some F1 hybrids of CBG and *A. capillaris* are reported to fail to flower while others have 41% mean pollen fertility, which was characterized as "low fertility" by the authors (Edgar and Forde 1991). Christoffer (2003) characterized these same results as demonstrating "moderate" pollen fertility, while Wipff (2002) said they demonstrated "poor" pollen fertility. Similar results have been reported in older studies on other CBG x *A. capillaris* F1 hybrid populations (F1 pollen fertility ranging from 0% to 37%, with an average of 13%, compared to 70% - 100% pollen fertility in the presumed parent populations) (Jones 1956a; Bradshaw 1958; Widén 1971). A more recent experiment found 20% pollen fertility in hybrids of ASR368 CBG and *A. capillaris*, compared to 90% fertility in the parental species (Zhao *et al*. 2007). Bradshaw (1958) (1958) points out that pollen fertilities of 20% imply a much lower (5% or less) capacity to produce successful offspring, but also notes that he observed a small percentage of likely F2 and backcross progeny in his study of a natural F1 hybrid population. Davies reported a low number of seedlings produced during open pollination of F1 hybrids (roughly 25% the number produced by each parent species) and a very low number produced in crosses between F1 hybrids (Davies 1953; Bradshaw 1958). A more recent report specifically examining the ability of open-pollinated F1 hybrids to produce viable offspring in backcrosses with their non-CBG parent under optimal conditions found that glufosinate resistant CBG x *A. capillaris* F1 hybrids demonstrated wide variability in both female and male fertility, with large numbers of viable offspring produced in some instances (Belanger *et al*. 2003a). Although the experiment was not designed to compare the level of fertility of the F1 hybrids to that of the parent species, it

clearly demonstrated that CBG x *A. capillaris* F1 hybrids can be fertile. Similarly, in a small study, Zhao *et al* (2007) reported that 9 of 11 seeds produced in a forced backcross of ASR368 CBG x *A. capillaris* males to *A. capillaris* females were able to germinate. However, only 1.9 seeds were produced per inflorescence, indicating that the F1 hybrids are not highly fertile. The viability and vigor of the resulting seedlings was not examined.

Less extensive data is available for other hybrid combinations. F1 hybrids from crosses of CBG with *A. gigantea* have been reported as being nearly as fertile as the parent species in open-pollination, but having lower fertility in F1 hybrid crosses (roughly 10% of open-pollination fertility) (Davies 1953), having 0% - 30% pollen fertility (Jones, 1956b), or having very poor pollen fertility with no seed set (Widén 1971). These results have been described as demonstrating that the F1 hybrids were "quite fertile," or have "poor to moderate" pollen fertility (Christoffer 2003). Belanger *et al*. (2003a) reported that open-pollinated F1 hybrids grown in optimal conditions demonstrated wide variability in both female and male fertility, with in some instances large numbers of viable offspring produced upon backcrossing to either parent. The same report found that F1 hybrids of CBG and *A. castellana* were also fertile, but had an extremely poor ability to serve as pollen donors in backcrosses with *A. castellana*, while F1 hybrids of CBG and *A. canina* had quite poor fertility and almost no ability to serve as pollen donors in backcrosses with *A. canina* (Belanger *et al*. 2003a). In the study by Zhao *et al*. (2007), F1 hybrids between ASR368 CBG and *A. gigantea*, *A. idahoensis* and *P. monspeliensis* exhibited from 20% - 34% pollen viability, but produced very few or no (*P. monspeliensis)* seed upon back-crossing, and none of the seed germinated. Finally, in a recent report, Zapiola and Mallory-Smith (2012) reported on a single F1 hybrid of ASR368 CBG and *P. monspeliensis*, finding that 16% of florets produced viable seed upon self-pollination in bagged panicles in a greenhouse experiment.

The generally low level of fertility observed in the F1 hybrids reported above is consistent with reports of a high level of meiotic irregularities in several of these F1 hybrids, which would be expected to lead to aneuploidy in backcross or F2 hybrid plants as observed by Jones (Jones 1956a, 1956b; Bradshaw 1958; Zhao *et al*. 2007). Indeed, Jones (1956b) reported that even though *A. capillaris x A. gigantea* F1 hybrids appeared to have a greater level of pollen fertility than the CBG hybrids discussed above, and were as fertile as their parents in terms of seed set, the resulting F2 hybrids were largely anueploid and showed very low fitness. He noted that no aneuploids had been detected in natural populations, as also noted by Widen (1971), suggesting that F2 hybrids are rare. Moreover, although fertility of hybrid plants often can be restored upon backcrossing (Zemetra *et al*. ; Rieseberg and Wendel 1993). Bradshaw (1958) argues that it is unlikely that fertility of CBG hybrids will be restored upon backcrossing, and thus that the possibility of gene introgression from one species to another is likely to be even lower than the estimated 5% or less viable F1 hybrid offspring, "since the barrier of sterility will act in a geometric fashion if it affects several generations." This is consistent with the report of Jones (1956a) that F2 hybrids of *A. capillaris x A. gigantea* exhibited 53% - 63% stainable pollen, only slightly greater than the 41% - 55% observed in the F1 hybrids (APHIS could find no other reports of F2 hybrid fertility).Nonetheless, given that F2 hybrid and backcross offspring of CBG x *A. capillaris* have been observed in nature

(Bradshaw 1958) and F2 hybrid and backcross offspring of CBG with several other *Agrostis* species have been observed in experimental studies (Bjorkman 1954; Jones 1956b; Belanger *et al*. 2003a) there remains potential for successful gene introgression from CBG to a sexually compatible species. Therefore, APHIS assessed whether the engineered glyphosate resistance trait could render F1 hybrids or sexually compatible species that have acquired the trait from GRCBG more difficult to control.

Potential for enhanced weediness of recipients after hybridization and/or introgression

Sexually compatible relatives of CBG are reported to be weeds of low to moderate importance in various settings (Table 2). The most significant weed risks are from *A. capillaris, A. gigantea,* and *A. exerata* to grass seed production fields in Idaho and Oregon. (*A. idahoensis* and *A. scabra* are also reported to pose a moderately important weed risk to grass seed production fields, but they appear to be less likely to hybridize with CBG.) *P. monspeliensis* is a moderately important weed in potato, sugarbeet, corn, and alfalfa in Idaho and to a lesser extent in Oregon, it is a moderately important weed in wheat California and to a lesser extent in Oregon, and a weed of low importance various vegetable and fruit crops in California (Banks *et al*. 2004; Canevari *et al*. 2006).

CBG and many of its sexually compatible relatives are capable of vegetative spread via stolons (CBG, *A. canina, A. capillaris*) or rhizomes (*A. capillaris, A. castellana, A.exarata, A. gigantea, A. pallens*) and the spread of plant fragments that move via water to new locations (Davies 1953; Widén 1971; Dore 1980; Kik *et al*. 1990b; Edgar and Forde 1991; MacBryde 2006; Harvey 2007). Hybrids of CBG with *A. capillaris, A. gigantea,* and *P. monspeliensis* are perennial and stoloniferous (Bradshaw 1958; Widén 1971; Edgar and Forde 1991; Zapiola *et al*. 2008). APHIS therefore reviewed information on the vegetative vigor and spread of interspecific hybrids of CBG.

There are several reports that naturally occurring hybrid populations of CBG and *A. capillaris* are vegetatively vigorous, long-lived, and can outcompete both parental species in certain habitats (Bradshaw 1958; Widén 1971; Edgar and Forde 1991). Natural hybrids of CBG with *A. gigantea* and *P. monspeliensis* are also reported to be vegetatively vigorous, though the vigor of the latter appears to vary, perhaps depending on the ecotype of the parent (Bradshaw 1975; Banks *et al*. 2004; MacBryde 2006). In all cases, the hybrid populations examined were long-established and thus had been selected for their persistence. Importantly, after nearly a century of CBG seed production in Oregon, there are no reports that hybrids between CBG and any of these species are more competitive, prevalent, or damaging than the parental species.

In a recent series of experiments comparing the vegetative vigor of F1 hybrids between glufosinate resistant CBG and *A. canina, A. capillaris, A. castellana,* and *A. gigantea,* Hart *et al.* (2009) reported that most of the hybrids were no more competitive than their parental species, and in many cases were less competitive, when grown in Kentucky Bluegrass turf and in roadside turf of mixed composition. However, the CBG x *A. gigantea* hybrids were more competitive than *A. gigantea* in both types of turf. Hart *et al.* (2009) argue that this result is likely due to the morphology of the hybrid plants, which combined the stoloniferous characteristic of CBG with the large leaves of *A. gigantea*,

rather than to the glufosinate resistance trait. These experiments do not resolve whether the transgene had any effect on vegetative vigor in either CBG or the hybrid since no assessment of non-transgenic CBG or F1 hybrids was conducted. As discussed in section F, in other experiments ASR368 CBG showed no greater vigor and no differences in other plant growth, reproduction, or persistence characteristics than non-transgenic CBG varieties. Scotts/Monsanto (2015a) found either no difference or a decrease in the vegetative vigor of ASR368 CBG F1 hybrids with *A. capillaris, A. idahoensis, A. pallens,* and *P. monspeliensis* relative to nontransgenic parents (Tables IX-6 and IX-7). Hybrids of ASR368 CBG and *A. gigantea* exhibited more vegetative growth than *A. gigantea* (but not than non-transgenic CBG) in a poly-house experiment, but not in a field experiment (Tables IX-6 and IX-7). In these experiments there was no assessment of the vigor of non-transgenic hybrids. The weight of evidence suggests that any interspecific F1 hybrids formed between GRCBG and sexually compatible species could persist in amenable habitats and may spread vegetatively. However, with the possible exception of *A. gigantea* hybrids, their vigor and spreading ability will be no greater than that of the parental species.

The same herbicides that control CBG can also control the other bentgrass species, although *A. castellana, A. gigantea* and possibly *A. canina* may be somewhat more resistant than CBG to the ACCase inhibitors (clethodim, fluazifop-P, sethoxydim) and perhaps other herbicides, but not more resistant to glyphosate (Mueller-Warrant 2003; Hart *et al*. 2005; Peachey 2016). For instance, across a range of herbicides tested, CBG and *A. capillaris* required and average of 3.1 herbicide treatments for complete control, while *A. canina, A. castellana,* and *A. gigantea* required an average of 3.5 – 3.6 treatments (Mueller-Warrant 2003). Of these three species, *A. gigantea* is the most likely to form hybrids with CBG, is the only one known to pose a weed risk of any importance, and is the only one to demonstrate potentially greater vigor than either parent species. Thus, with the potential exception of *A. gigantea*, the analysis and conclusions of the impact of the *cp4 epsps* transgene and glyphosate resistance on the ability to control CBG also apply to any F1 hybrids formed with other *Agrostis* species and any subsequent generations. In the highly unlikely event that glyphosate resistant hybrids or subsequent generations did establish in grass seed production fields, they are unlikely to produce fertile seed. Moreover, since the seed size of other *Agrostis* species is similar to that of CBG (Darris and Bartow 2006; Stahnke *et al*. 2010), any viable seed produced would be extremely unlikely to be present as an impurity in grass seed.

For control of *P. monspeliensis* in Oregon and Idaho, clethodim and sethoxydim have the same effectiveness rating as glyphosate and can be used in alfalfa, potato, and sugarbeet, but not wheat or corn (except sethoxydim resistant corn) (DiTomaso et al. 2013; US-EPA 2015f, 2015g; Felix 2016; Hulting and Morishita 2016(Prather, 2016b #426; US-EPA 2016f). Clodinafop, fenoxaprop, and pinoxaden all provide good control and can be used in wheat, although clodinafop is not registered in California (Singh 2009; UC Pest Management Guidelines 2009; US-EPA 2009; Tagour *et al*. 2011; US-EPA 2013c, 2014c). Sethoxydim and/or fluazifop-P (which provides somewhat less control of *P. monspeliensis*) can be used on all of the vegetable and fruit crops in California listed by Banks *et al* (2004) (DiTomaso *et al*. 2013; US-EPA 2015g, 2015h, 2016g).

Conclusion

Scotts/Monsanto has no intention to commercialize or further propagate ASR368 CBG. Thus, the only opportunity for gene flow or introgression to sexually compatible species is from escaped ASR368 CBG and any feral GRCBG that persist in the environment. Based on the analysis above and the current sparse distribution of GRCBG, the frequency of such plants will be very low, and it is highly unlikely that the *cp4 epsps* transgene will introgress into wild or weedy species present in the natural environment. Since the *cp4 epsps* transgene does not appear to be associated with a detectable fitness cost in ASR368 CBG, if glyphosate resistant hybrids or subsequent generations do become established in the environment, they may persist in low numbers unless efforts are taken to eradicate them. If exposed to glyphosate they would have a fitness advantage compared to non-glyphosate resistant plants and could expand in number due to locally decreased competition from other plants. Alternatives to glyphosate exist for use against all of the sexually compatible relatives in the various settings where they pose weed problems. These alternative herbicides are generally effective, but as with GRCBG, any hybrid plants that do persist along irrigation ditches and canals or in grass seed production fields are likely to be somewhat more difficult to control than their glyphosate sensitive parental species. However, given the very low frequency with which hybrid plants are expected to form, the availability of alternative herbicides and other methods for management, and the very low level of hybrid fertility, APHIS concludes that any adverse consequences of gene flow from ASR368 CBG or feral GRCBG to wild or weedy species in the United States and its territories are extremely unlikely.

H. Potential Changes to Agriculture or Cultivation Practices

For most petitions for nonregulated status under the regulations found at 7 CFR part 340, APHIS assesses whether significant changes to agricultural or cultivation practices from adoption of the GE organism are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

However, in the subject petition for a determination of nonregulated status for ASR368 CBG, the developers have stated that they do not intend to commercialize ASR368 CBG nor license it to others for commercialization. Accordingly, APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of ASR368 CBG since it will not be commercialized or grown; therefore, no impact on plant diseases or pests or their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which ASR368 Creeping Bentgrass Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into ASR368 CBG to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Droge *et al*. 1998). Keese (2008) recently reviewed the literature regarding potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention. Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution.

Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

The ASR368 CBG has been engineered with the EPSPS gene from the bacterium *Agrobacterium sp.* strain CP4, the chloroplast transit peptide from the *Arabidopsis thaliana*, as well as non-coding regulatory DNA sequences derived from rice, maize, *Agrobacterium tumefaciens* and CaMV. Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (Keese 2008).

Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer 2008; Keese 2008) and HGT between plants and fungi is extremely rare (Richards *et al*. 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese 2008; Zhu *et al*. 2011; Acuna *et al*. 2012).

Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of ASR368 CBG is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood *et al*. 2001; Kaneko *et al*. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese 2008). Second, in cases where

review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin *et al*. 2001; Brown 2003; EFSA 2009). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, both the FDA (1998) and the European Food Safety Authority (EFSA 2009) have evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is very rare or remote.

Potential for horizontal gene transfer to viruses

As mentioned above, ASR368 CBG has been engineered with sequences derived from the plant virus CaMV. APHIS considered whether horizontal transfer of DNA from the ASR368 CBG to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. This issue has been considered before by other science review panels and government regulatory bodies (EPA-FIFRA-SAP 2006; Keese 2008).

HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g. geminiviruses which replicate in the nucleus) (Frischmuth and Stanley 1998) and RNA viruses (which typically replicate in the cytoplasm); however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in transgenic plants infected with related viruses are similar to recombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo *et al*. 2008).

Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morroni *et al*. 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, including in the CaMV 35 promoter, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the U.S. (Fuchs and Gonsalves 2007).

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (51 FR 23302) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr *et al*. 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer 2007). Recently, (Yoshida *et al*. 2010) through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant. According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS 2016f). More recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi *et al*. 2012) and 24 –41% of mitochondrial (Xi *et al*. 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of years ago. Furthermore in ASR368 CBG, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome.

If ASR368 CBG becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from the GE plant. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis APHIS therefore concludes that HGT of the new genetic material inserted into ASR368 CBG to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, public comments in response to Federal Register notices concerning this petition, and other relevant information to assess the plant pest risk of the ASR368 CBG compared to the unmodified variety from which it was derived. APHIS concludes that the ASR368 CBG is unlikely to pose a plant pest risk based on the following findings.

- Scotts/Monsanto has no intention to commercialize or further propagate ASR368 CBG in the future. Further, Scotts and Monsanto will not grant a license to or otherwise allow other entities to obtain, use, or propagate such plants (Scotts/Monsanto 2015a).

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in ASR368 CBG because it was developed with biolistic transformation protocols, it contains a single stable DNA insertion with no unintended sequence rearrangement, and none of the inserted sequences from plant pests encode a plant pest or infectious agent.

- No increase in plant pest risk was identified in ASR368 CBG due to expression from the inserted genetic material of new proteins or changes in metabolism or composition because the CP4 EPSPS protein is structurally similar and functionally identical to endogenous plant EPSPS enzymes, except for its insensitivity to glyphosate, and there are no substantive compositional differences between ASR368 CBG and conventional CBG.

- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in ASR368 CBG compared to the nontransgenic counterpart or other comparators in field trials. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that ASR368 CBG is more susceptible to pests or diseases**.** Therefore no plant pest effects are expected on ASR368 CBG, and ASR368 CBG is unlikely to differ from conventional CBG in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

- Exposure to and/or consumption of ASR368 CBG are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of the safety of the protein CP4 EPSPS, observations from multi- year U.S. field trials looking for adverse non-target interactions with the use of ASR368 CBG and the past evaluations of the impact of the EPSPS protein within approved petitions.

- ASR368 CBG (or feral CBG that acquires the glyphosate resistance trait) is unlikely to be weedier than conventional varieties CBG based on its observed agronomic characteristics, the weediness potential of the crop, and current management practices available to control GRCBG as a weed. GRCBG plants may be somewhat more difficult to control than glyphosate sensitive CBG in riparian habitats, grass seed production fields, and some hayfields and pastures, but can still be managed using a variety of currently available methods, including mechanical and cultural methods and alternative herbicides. GRCBG is unlikely to pose a significant weed problem and any adverse consequences from the escape and persistence of GRCBG are unlikely.

- ASR368 CBG is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. or its territories. Gene flow, hybridization and/or introgression of inserted genes from escaped or feral GRCBG to other sexually compatible relatives with which they can interbreed is not likely to occur since GRCBG is rare in the environment and will not be cultivated in the future. If gene introgression does occur, the new phenotype conferred by genetic engineering is not likely to increase the weediness of hybrid plants or any of these compatible relatives. The new phenotype may make these relatives somewhat more difficult to control, but they can still be managed using a variety of currently available methods and alternative herbicides. Glyphosate resistant sexually compatible relatives are unlikely to pose a significant weed problem and any adverse consequences from gene flow from GRCBG to wild or weedy species in the United States and its territories are highly unlikely.

- Significant changes to agricultural or cultivation practices of CBG (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of the ASR368 CBG will not occur since Scotts/Monsanto do not intend to commercialize or further propagate such plants in the future.

- Horizontal gene transfer of the new genetic material inserted into ASR368 CBG to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

K. References

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