

Escape and establishment of transgenic glyphosate-resistant creeping bentgrass *Agrostis stolonifera* in Oregon, USA: a 4-year study

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Summary

1. Gene flow from transgenic crops to feral populations and naturalized compatible relatives has been raised as one of the main issues for the deregulation of transgenic events. Creeping bentgrass, *Agrostis stolonifera* L., is a perennial, outcrossing grass that propagates by seeds and stolons. Transgenic Roundup Ready® glyphosate-resistant creeping bentgrass (GRCB), which is currently under USDA-APHIS regulated status, was planted in 2002 on 162 ha within a production control area in Oregon, USA.

2. We conducted a study to assess transgene flow from the GRCB fields. A survey within and around the production control area was performed during the year when the GRCB fields produced seed and for 3 years after the fields were taken out of production. Transgene flow was determined by testing creeping bentgrass and its relatives for expression of the glyphosate resistance transgene.

3. While GRCB seed-production practices were strictly regulated, evidence of transgene flow was found in all years. In 2006, 3 years after the transgene source fields were taken out of production and a mitigation programme was initiated, 62% of the 585 creeping bentgrass plants tested *in situ* were glyphosate-resistant (GR). Our results document not only the movement of the glyphosate resistance transgene from the fields, but also the establishment and persistence of high frequencies of GR plants in the area, confirming that it was unrealistic to think that containment or eradication of GRCB could be accomplished.

4. Synthesis and applications: These findings highlight the potential for transgene escape and gene flow at a landscape level. The survey provides empirical frequencies that can be used to design monitoring and management methods for genetically engineered (GE) varieties of outcrossing, wind-pollinated, perennial grasses and to evaluate the potential for coexistence of GE and non-GE grass seed crops. Such information should also be used in the decision-making process for authorization of field trials and deregulation of genetic engineering events.

Key-words: *Agrostis*, coexistence of genetically engineered crops, CP4 EPSPS, gene flow, genetically modified crops, genetic engineering, glyphosate resistance, glyphosate-resistant crops, transgenic crops.

Introduction

Gene flow from genetically engineered (GE) crops has been raised as a major concern when considering the deregulation of new genetic engineering events in general (Giddings 2000; Ellstrand 2001, 2003a, 2003b; Andow & Zwahlen 2006). Gene flow is defined as the change in gene frequency due to

movement of gametes, individuals or groups of individuals from one place to another (Slatkin 1987).

Creeping bentgrass, *Agrostis stolonifera* L., is the most widely used cool-season grass for high-quality golf course tees, greens and fairways (Turgeon 1996; Wipff & Fricker 2001; Warnke 2003), where other grasses are the main weed problem (Gange, Lindsay & Ellis 1999; Belanger *et al.* 2003). Glyphosate is a broad-spectrum, non-selective herbicide; therefore, transgenic glyphosate-resistant creeping bentgrass

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(GRCB) would allow a more flexible and effective way of controlling problem weeds.

The *CP4 EPSPS* gene, isolated originally from *Agrobacterium* sp. strain CP4, which encodes the CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) protein that confers resistance to glyphosate herbicide, was incorporated via DNA recombination techniques into a creeping bentgrass cultivar (event ASR368, The Scotts Company, Marysville, OH, USA; Monsanto Company, St. Louis, MO, USA). Although a petition (APHIS petition no. 03-104-01p) was submitted in April 2003 requesting the deregulation of Roundup Ready® GRCB, this GE grass is still under USDA-APHIS regulated status. Glyphosate-resistant creeping bentgrass was the first GE crop in the USA for which an Environmental Impact Statement was requested.

The main concerns raised regarding the deregulation of GRCB were the contamination of non-GE grass seed crops (especially of seed lots to be exported to countries with zero tolerance for GE organisms); the introgression of the *CP4 EPSPS* gene via pollen flow into feral and naturalized compatible weed species; and the control of GRCB volunteer plants. Another concern was the evolution of glyphosate-resistant weeds due to the increased selection pressure of repeated glyphosate applications on GRCB fields.

Although creeping bentgrass is a cosmopolitan, phenotypically plastic and evolutionarily adaptive species, it is rarely invasive in natural or seminatural areas (MacBryde 2006). Nevertheless, creeping bentgrass has several characteristics that increase the potential of transgene flow, establishment, introgression and persistence into nearby compatible sympatric populations. First, it is an outcrossing, wind-pollinated, perennial grass that propagates sexually by seed and vegetatively by stolons. Second, seeds of creeping bentgrass are small, $\approx 13\,500$ seeds g^{-1} (AOSA 2002), and thus can be easily dispersed. Creeping bentgrass seed can germinate soon after dispersal, but also persist in the seed bank where they germinate for at least 4 years (unpublished data). The average seed yield for creeping bentgrass in Oregon, USA, is 600 kg ha^{-1} (USDA-NASS 2006), representing 8.1×10^9 seeds ha^{-1} . Finally, creeping bentgrass is an allotetraploid, and polyploidy is generally associated with a greater probability of producing fertile interspecific hybrids (Warnke 2003).

Agrostis, one of the most difficult and complicated grass genera from a taxonomic point of view, is a cosmopolitan genus with ≈ 200 species worldwide that exists in a broad variety of habitats (Hitchcock 1971; Hitchcock & Cronquist 1973; Harvey 1993; Warnke 2003). Creeping bentgrass, redtop (*A. gigantea* Roth), colonial bentgrass (*A. capillaris* L.), dryland bentgrass (*A. castellana* Boiss. & Reuter), velvet bentgrass (*A. canina* L.) and brown bentgrass (*A. vinealis* Schreber) form a complex of interpollinating, cross-compatible species. Sympatric populations may contain interspecific hybrids with varying degrees of pollen fertility and seed set (Wipff & Fricker 2001; Belanger *et al.* 2003). Redtop, colonial, dryland and velvet bentgrass, as well as spike bentgrass (*A. exarata* Trin.), occur in Oregon. Creeping bentgrass has also been reported to form intergeneric hybrids with rabbitfoot grass (*Polypogon monspeliensis*

(L.) Desfontaines) and water bent (*P. viridis* (Gouan) Breistr.), both of which occur in Oregon (Wipff & Fricker 2001).

In 2002, the Oregon Department of Agriculture established a 4500-ha seed production control area at Jefferson County, 2.5 km north of Madras (44°38'1" N, 121°7'42" W), OR, USA (Oregon Administrative Rule 603-052-1240), and 162 ha were planted with GRCB. The control area is an elevated plateau with an elevation of about 720 m and has a 270 m drop to the Deschutes River to the north-west and a more moderate drop to Mud Springs Creek to the east. The other limits are mainly local roads to the north-east, and USA Highway 26 to the south-west. The region is characterized by a high desert climate with average maximum and minimum temperatures of 31 and 7, and 6 and -6 °C for July and January, respectively. The average rainfall in the area is 246 mm year⁻¹. However, because the control area is part of the North Union Irrigation District, it has mesic areas such as canal banks, irrigation and drainage ditches and ponds that are ideal for establishment of creeping bentgrass seedlings. The main crops planted are Kentucky bluegrass (*Poa pratensis* L.), rough bluegrass (*Poa trivialis* L.), carrot (*Daucus carota* L. ssp. *sativus* (Hoffmann) Arcang.), onion (*Allium* spp.) and garlic (*Allium sativum* L.), all for seed production, and alfalfa (*Medicago sativa* L.) for hay. The control area has multiple growers, each with their own weed management programme. The surrounding area consists of grasslands and arid landscapes to the north-west and east, and irrigated agricultural land to the south.

The 162 ha of GRCB planted in 2002 were distributed among eight fields within the control area. In 2003, after seed harvest, the eight GRCB fields were taken out of production. An additional 2.4-ha GRCB field was planted in 2003, flowered and produced seed in 2004. Production practices within the control area were strictly regulated and monitored to minimize gene flow from GRCB seed fields. Some of the control area requirements were: (1) non-GE *Agrostis* spp. could not be planted, grown or handled within the control area; (2) GRCB was to be located more than 400 m away from any bentgrass field outside the control area; (3) field borders, ditch banks and roadsides within 50 m of the GRCB fields were to be kept free of *Agrostis* spp.; (4) waterways leaving GRCB fields were to be kept free of *Agrostis* spp. for 50 m; (5) GRCB seed was to be transported in enclosed containers; (6) equipment such as swathers and balers had to be cleaned thoroughly before leaving the control area, and combines were to be used exclusively for GRCB and cleaned and fumigated thoroughly after use; (7) all straw from GRCB fields was to be burned within the control area or processed in a way that devitalized the creeping bentgrass seeds; (8) GRCB stands were to be removed by application of effective herbicide after watering to promote growth, followed by shallow tillage; (9) GRCB volunteers were to be controlled in the subsequent crop (Oregon Administrative Rule 603-052-1240). At the time of seeding, The Scotts Company further increased the distance from the GRCB fields to be kept free of *Agrostis* spp. to 300 m. Although not part of the original control area rules, fields were fumigated with metham sodium after the removal of the GRCB stands.

Knowledge of the extent of transgene flow over time at a landscape level is very limited for outcrossing grasses (Snow 2002; Tolstrup *et al.* 2003). Therefore, we conducted a 4-year survey to evaluate gene flow from the GRCB fields, to test our hypothesis that the preventive measurements were not enough to contain the transgene. The objective of our survey was to determine the presence and distribution of creeping bentgrass and its compatible relatives carrying the *CP4 EPSPS* transgene in the area, as a measure of the establishment and introgression of the transgene in the feral, non-GE populations.

Materials and Methods

AREA AND DISTANCE SURVEYED

A 4-year study to assess gene flow from the 162 ha GRCB fields planted in the control area was conducted during late spring and summer, starting in 2003. Banks of irrigation canals, ditches and ponds, roadsides and pipeline sides, which were potential habitats for creeping bentgrass and its compatible relatives, were walked to scout for plants (Figs S1–S3 in Supplementary Material). Production fields were not surveyed. In 2003, a total of ≈ 80 km of irrigation canal, ditch and pond banks, roadsides and pipelines were surveyed, mainly in the area 300 m around the GRCB production fields. In 2004, the area surveyed was expanded within the control area in general, as well as outside the control area to the north-east, to study the consequences of a documented wind event. The total distance scouted in 2004 was ≈ 130 km. The same general procedure was followed in the two following years. Although some sites were revisited, the area surveyed was extended up to a 5 km radius outside the control area. The distance walked in 2005 and 2006 was ≈ 200 km each year.

PLANT IDENTIFICATION

Correct identification of *Agrostis* species based on morphological characteristics is difficult (Warnke 2003). Therefore the main effort of the surveys was concentrated from mid-July until the end of September, when visible panicles aided in identification of species. Also, panicles with seeds could be collected to determine the incidence of gene flow via pollen.

GLYPHOSATE-RESISTANT PLANT DETECTION

Gene flow was determined by the occurrence of individuals of creeping bentgrass and its compatible relatives that contained the *CP4*

EPSPS transgene. Plants found were tested *in situ* for the presence of the *CP4 EPSPS* protein using Trait✓ RUR TM strips (Strategic Diagnostics, Newark, DE, USA). A leaf sample ≈ 2 –7 mg, generally a 1–2 cm segment, was placed in a 1.5- or 2-ml centrifuge tube with ≈ 0.5 ml distilled water. The tissue was homogenized using a disposable plastic stirrer, then a Trait✓ RUR TM strip was placed in the tube. After a maximum of 5 min, plants were categorized as GR or susceptible. The reliability of the Trait✓ RUR TM strips in detecting the *CP4 EPSPS* protein was determined previously with PCR and sequencing techniques, and they were found to be 100% accurate in GRCB (Watrud *et al.* 2004). At sites with many plants present, tissue from up to 10 plants was pooled and tested in a single tube. If the test was positive for resistance then 40% of the plants were tested individually; if all proved to be GR, the rest of the plants in the patch were considered 'putative' GR, even though not all plants were tested.

MAP CONSTRUCTION

Plants were geo-referenced using a hand-held eTrex Legend global positioning system (GPS) (Garmin, Olathe, KS, USA). When patches of several creeping bentgrass or redtop plants were found, plants were counted to the extent that it was possible to identify individual plants, but only one GPS location was recorded. Therefore a single map point may represent more than one plant. When it was impossible to determine if a patch was a large single plant or several smaller plants, the patch was recorded as a single plant. Maps were prepared using ESRI ArcMAP 9.1 (ESRI, Redlands, CA, USA).

Results

In the 2003 *in situ* survey, none of the 57 plants tested near the GRCB production fields carried the *CP4 EPSPS* transgene. Within those 57 plants, 14% were feral creeping bentgrass, 84% were redtop and 2% were rabbitfoot grass (Table 1). However, 0.376% of the seeds from creeping bentgrass and redtop panicles collected in 2003 resulted in GR seedlings when tested in the glasshouse (Mallory-Smith, Butler & Campbell 2005) (Fig. 1).

In 2004, GR plants were found along canals and irrigation ditches throughout the control area. Creeping bentgrass plants were found in locations where they were not present in 2003 (Fig. 2). A total of 300 plants were tested *in situ* in 2004, 49% of which were identified as creeping bentgrass. Redtop and rabbitfoot grass accounted for 37 and 10% of the total

Table 1. Number of glyphosate-susceptible (S), glyphosate-resistant (R) and putative glyphosate-resistant (P) plants for each species found *in situ* in 2003–06

Species	2003*			2004			2005			2006		
	S	R	P†	S	R	P	S	R	P	S	R	P
<i>Agrostis stolonifera</i>	8	0	0	10	138	270	447	521	437	225	360	427
<i>Agrostis gigantea</i>	48	0		110	0		249	0		374	0	
<i>Polypogon monspeliensis</i>	1	0		29	0		6	0		45	0	
<i>Agrostis</i> spp.	0	0		10	0		65	0		61	0	
Potential hybrid	0	0		3	0		1	1		3	5	
Total	57	0	0	162	138	270	768	522	437	708	365	427

*Due to limitations of the survey it is not appropriate to compare between years.

†Putative glyphosate-resistant: plant that was within a group of glyphosate-resistant plants but was not tested.

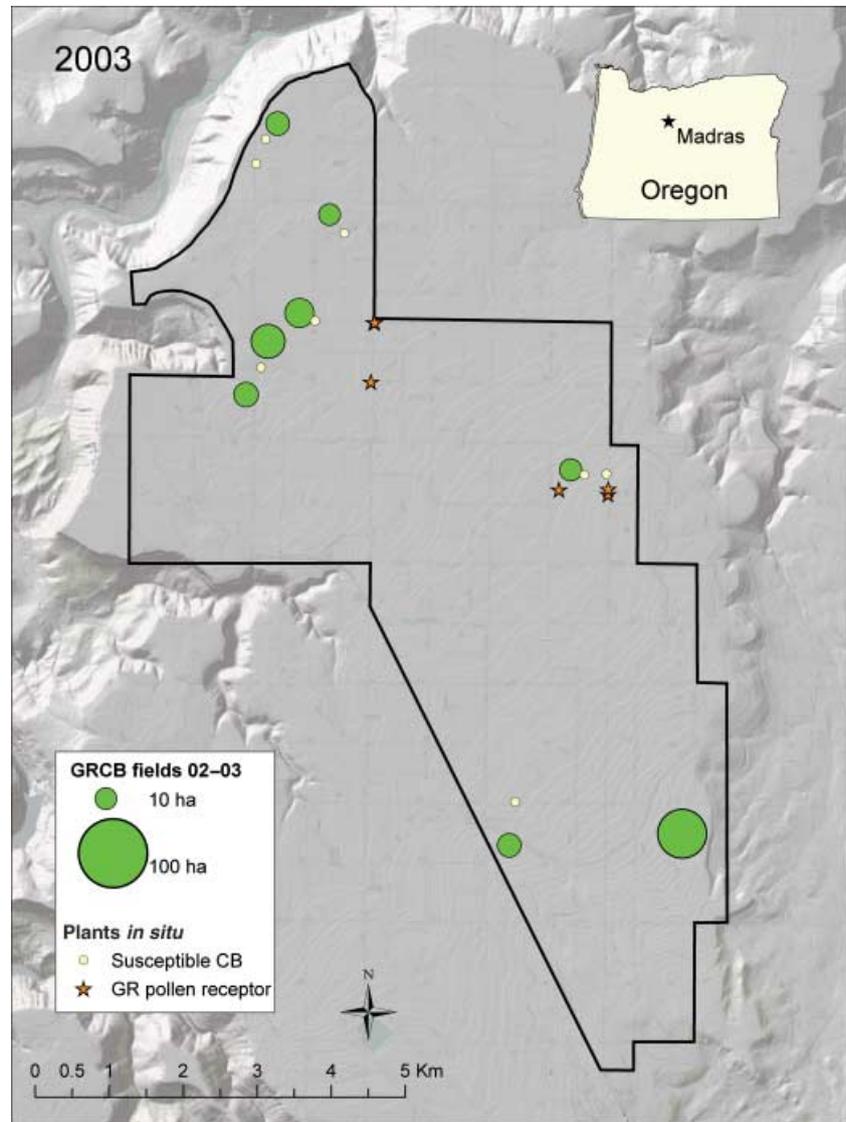


Fig. 1. Creeping bentgrass (CB) plants tested *in situ* in 2003. Location of transgenic glyphosate-resistant CB (GRCB) fields in 2003 (fields to scale but not true to shape), susceptible CB plants found *in situ* (a single point may represent more than one plant), and susceptible plants that produced glyphosate-resistant seedlings in 2003 (GR pollen receptor). Black line, limit of GRCB control area. Inserted map shows the location of Madras in relation to the state of Oregon, USA.

plants tested, respectively (Table 1). Due to the inability to identify some plants to species *in situ*, the remaining 4% of the plants were identified as *Agrostis* spp. or potential hybrids with creeping bentgrass. Although, overall, 46% of plants tested carried the *CP4 EPSPS* gene, 93% of the 148 creeping bentgrass plants tested were GR (Fig. 2) and a further 270 plants were designated as putative GRCB (increasing the possible percentage to 97.5% GR). None of the redtop, rabbitfoot grass, other *Agrostis* spp. or potential interspecific hybrid plants tested was GR. The most distant GRCB plant found was 1.9 km from the closest original GRCB production field. Based on the ≈ 130 km surveyed in 2004, the GR plant abundance was ≈ 1.1 plants km^{-1} (3.1 plants km^{-1} if putative GRCB plants are considered).

In 2005, a total of 1290 plants were tested *in situ*: 75% were identified as creeping bentgrass, 19.3% redtop, 0.5% rabbitfoot grass, and the remaining 5.2% was represented by *Agrostis* spp. and potential hybrids (Table 1). Overall, 40.5% of the plants tested were GR. The GR plants were identified as creeping bentgrass, except for one potential interspecific hybrid.

Of the 968 creeping bentgrass plants tested, 54% were GR (Fig. 3), or 68% if the 437 putative GRCB plants are considered. Creeping bentgrass plants were generally found close to or in the water, while redtop plants were generally found higher on the banks. The most distant GRCB plant found was 4.6 km from the closest original GRCB production field. The ≈ 200 km surveyed resulted in a GR plant abundance of ≈ 2.6 plants km^{-1} for 2005 (4.8 plants km^{-1} including putative GRCB plants).

In 2006, sites that were heavily infested with GRCB in 2005 were revisited to assess the persistence of GRCB. In addition, the survey area was extended to more distant areas outside the control area. A total of 1073 plants were tested for glyphosate resistance *in situ*. Of the 1073 plants tested, 55% were creeping bentgrass, 35% redtop, 4% rabbitfoot grass, and the remaining 6% were classified as *Agrostis* spp. or potential hybrids (Table 1). Overall, 34% of the total plants tested were GR. The GR plants were identified as creeping bentgrass, except for five potential interspecific hybrids. Of the 585 creeping bentgrass plants tested, 62% were GR (Fig. 4), or 78% if the 427 putative GRCB were included. In 2006, the most distant

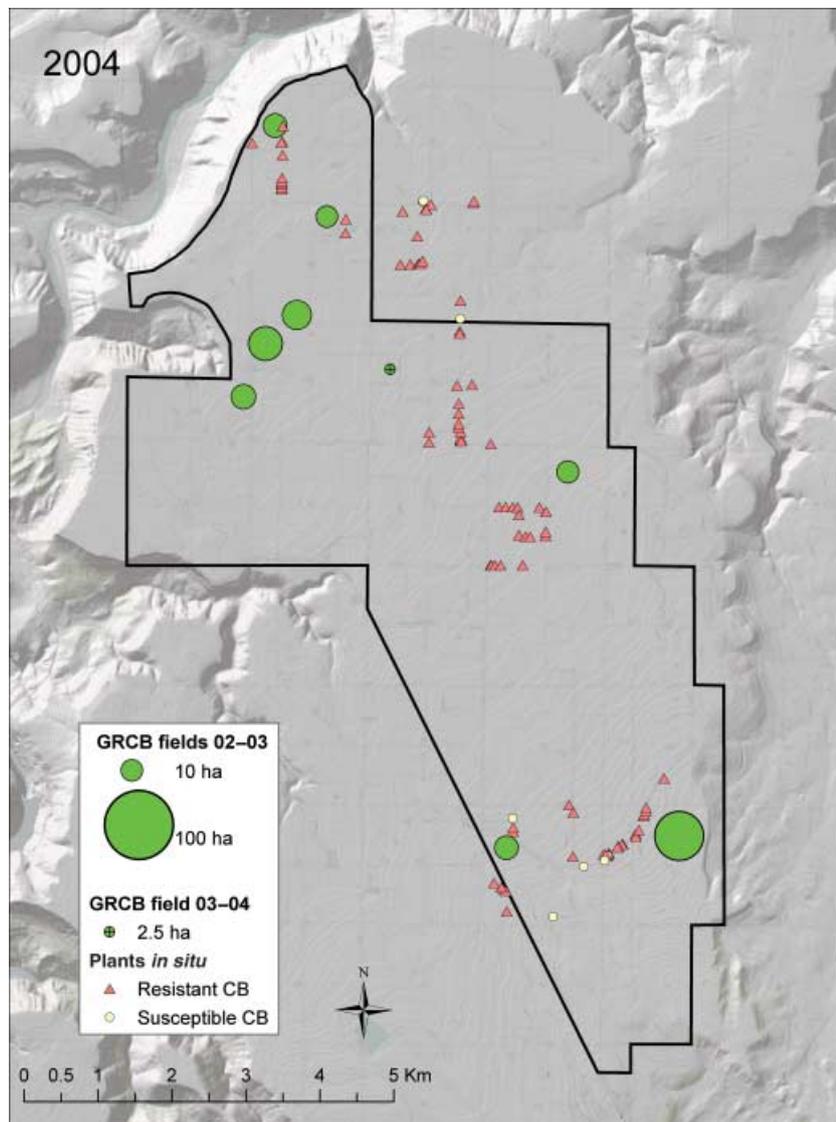


Fig. 2. Creeping bentgrass (CB) plants tested *in situ* in 2004. Location of transgenic glyphosate-resistant CB (GRCB) fields in 2003 and 2004 (fields to scale but not true to shape), and glyphosate-resistant and susceptible CB plants found *in situ* (a single point may represent more than one plant). Black line, limit of GRCB control area.

GRCB plant was 4.6 km away from an original GRCB field. For the ≈ 200 km surveyed in 2006, the GR plant abundance was ≈ 1.8 plants km^{-1} (4.0 GR plants km^{-1} if the putative GRCB plants are considered).

It is important to note that the area surveyed in 2005 and 2006 was considerably larger than that surveyed in 2004, which was in turn larger than the 2003 area. Although the distances walked in 2005 and 2006 were similar, and some previous sites were revisited, not all sites were the same. No direct comparison of total number of plants or plant density can be performed among years. Because of the regulated status of GRCB, USDA-APHIS required that The Scotts Company remove GRCB plants in the area each year, so we were unable to follow all plants over time. However, some sites had GR plants that persisted throughout the 3 years.

Discussion

The introduction of GRCB to the control area was a unique and novel starting point from which to evaluate transgene

flow from a regulated GE crop to its relatives at a landscape level. The results show that the *CP4 EPSPS* transgene escaped from the GRCB fields and continued to spread for 3 years after the fields were taken out of production. As we hypothesized, it was unrealistic to think that a transgene could be contained in an outcrossing, wind-pollinated, small-seeded, perennial crop, even with expanded isolation distances and stringent production practices. This fact has implications for the deregulation and production of GE crops in the future, especially those for pharmaceutical or industrial uses. It should be noted that although GRCB was introduced in the area in 2001, less than 1 ha was planted and seed was hand-harvested in 2002 (The Scotts Company, personal communication) and no GRCB plants were found *in situ* in 2003. We are therefore confident that the main *CP4 EPSPS* transgene source was the 162 ha planted to GRCB in 2002.

Although no plants were found *in situ* expressing the *CP4 EPSPS* gene in 2003, pollen-mediated transgene flow was found in the progeny of 2003 plants (Mallory-Smith *et al.* 2005). These findings are in accordance with Watrud *et al.*

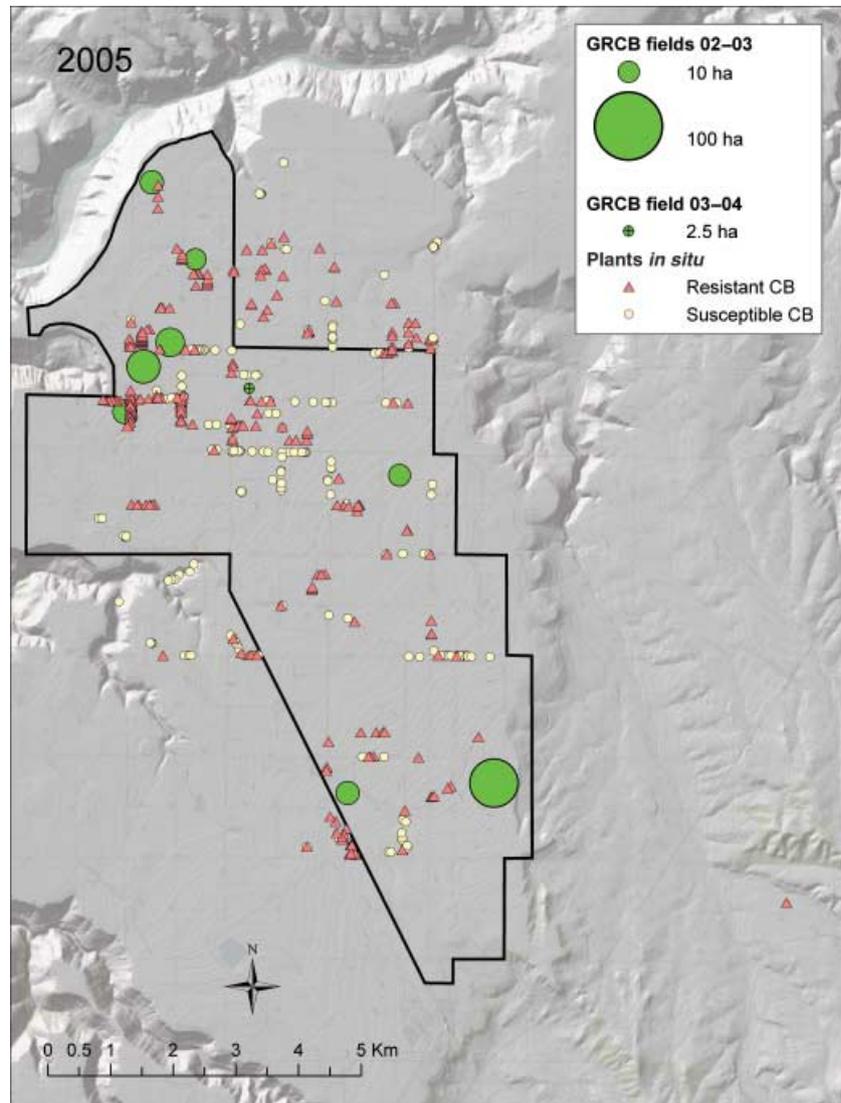


Fig. 3. Creeping bentgrass (CB) plants tested *in situ* in 2005. Location of transgenic glyphosate-resistant CB (GRCB) fields in 2003 and 2004 (fields to scale but not true to shape), and glyphosate-resistant and susceptible CB plants found *in situ* (a single point may represent more than one plant). Black line, limit of GRCB control area.

(2004), who reported *CP4 EPSPS* gene flow via pollen to susceptible sentinel and resident creeping bentgrass plants, as well as to resident redtop plants, within a year, mostly within 2 km of the control area perimeter and in the direction of prevailing north-westerly winds.

In August 2003, there was a documented strong north-westerly wind event in the production control area that moved seed and panicles from swathed windrows of the northernmost GRCB production field (local growers; The Scotts Company, personal communications). This wind storm is considered to be responsible for the presence of GR plants found south-east of that field, in places where no creeping bentgrass plants were identified before (Figs 2–4), implying gene flow via seed. Previous studies have reported the importance of wind events in determining the directionality of gene flow via pollen but not via seed (Giddings, Sackville Hamilton & Hayward 1997; Watrud *et al.* 2004; Halsey *et al.* 2005; Hoyle & Cresswell 2007). Because creeping bentgrass can easily propagate vegetatively (Carrier 1924), stolons also have to be regarded as a potential mechanism of gene flow in creeping bentgrass

(Dysart & Mallory-Smith 2006), with waterways likely to be an important route for both GRCB seed and stolon dispersal.

The means by which the *CP4 EPSPS* transgene moved (pollen, seed or vegetative propagules) is difficult to determine. Analysis of nuclear ribosomal ITS1-5.8S-ITS2 (ITS) and maternally inherited chloroplast *trnK* intron maturase (*matK*) gene trees of the nine GR plants found by Reichman *et al.* (2006) suggested that establishment resulted from both pollen-mediated intraspecific hybridizations and seed dispersal. Current research aims to clarify how the transgene moved in the hundreds of GR plants we found *in situ* during the 4 years of our survey.

The 46% of GR plants we found *in situ* in 2004 is orders of magnitude greater than the 0.04% in the 2004–05 samples tested by Reichman *et al.* (2006). The difference could be due to the fact that Reichman *et al.* (2006) surveyed exclusively in a 4.8-km zone beyond the control area, while our survey was conducted mainly inside the control area, and outside the control area downwind from the field where the reported wind event took place (Fig. 2). In addition, methodologies

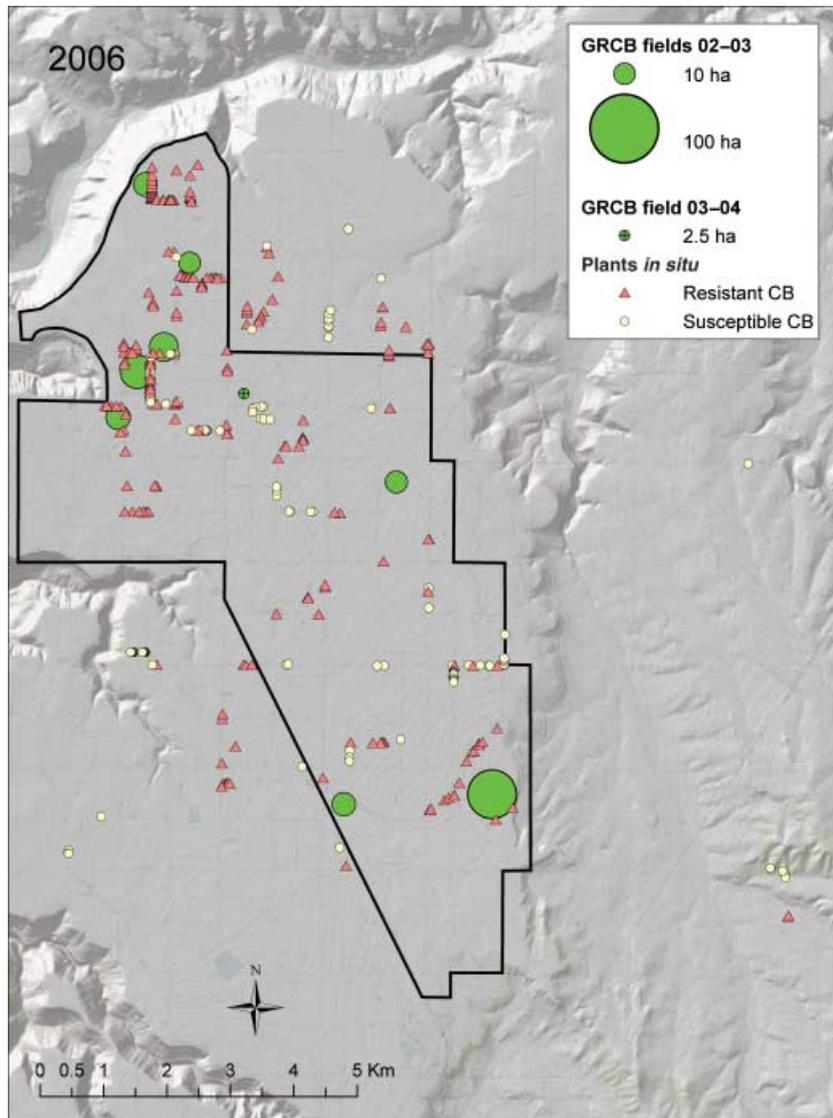


Fig. 4. Creeping bentgrass (CB) plants tested *in situ* in 2006. Location of transgenic glyphosate-resistant CB (GRCB) fields in 2003 and 2004 (fields to scale but not true to shape), and glyphosate-resistant and susceptible CB plants found *in situ* (a single point may represent more than one plant). Black line, limit of GRCB control area.

differed between the studies. Reichman *et al.* (2006) pooled between 40 and 50 leaf segments, each representing one plant, while we pooled a maximum of 10 leaf segments in a sample.

The fact that 62% of creeping bentgrass plants tested in 2006 were GR, even after an intense and extended mitigation programme had been initiated (and is still under way), supports the opinion of Marvier & Van Acker (2005) that elimination of escaped transgenes is unlikely to be feasible. It will be particularly difficult in cases such as GRCB (Fei & Nelson 2004; Carter *et al.* 2005), where the transgene apparently does not have a fitness cost (Ellstrand 2003b; Andow & Zwahlen 2006).

Unlike traits such as drought or salt tolerance and disease resistance, which could increase the fitness of the transgenic plant and result in an even greater frequency of gene flow and introgression, GRCB plants do not have a competitive advantage over non-GE plants unless glyphosate is applied (Belanger *et al.* 2003; Gardner, Danneberger & Nelson 2004). Glyphosate was sprayed on some of the canal and ditch banks in the area, which could have favoured the establishment and persistence of GRCB (Fig. S3). However, we think that the

high percentage of plants found in 2004 was due mainly to seed dispersion from the production fields during 2003.

In perennial species such as creeping bentgrass, one plant can contribute its transgene to the population for more than 1 year, increasing the possibility of introgression with feral creeping bentgrass and compatible native and naturalized species. Under standard agronomic production scenarios, creeping bentgrass is often maintained in production for up to 5 years. Therefore the standard practices would further increase the likelihood and frequency of transgene escape. Although we found GRCB at 4–6 km from the closest original GRCB field, it is not possible to know or predict how far the transgene has moved. Any extrapolation beyond the most distant point tested would be inappropriate (Ellstrand 2003a). In addition, because there were eight GRCB fields, it is not possible to identify which was the transgene source for any of the GR plants found. It is advisable that, if any other planting of a regulated genetic engineering event is authorized, it should be planted at a single site to facilitate the estimation of gene flow and of required isolation distances.

Agrostis spp. plants are plastic and variable in morphology, which makes it difficult to identify hybrids. Although we have not yet confirmed the presence of any interspecific hybrids *in situ*, GR plants originating from seeds collected from susceptible redtop plants have been identified in glasshouse screenings (Zapiola *et al.* 2007). Whether F_1 hybrids produce viable pollen and seed is unknown, but prior studies suggest that intra- and interspecific hybrids can have high fertility (Wipff 2002). Because we found plants with intermediate morphology, we believe it is only a matter of time until we confirm the first hybrid *in situ*.

Glyphosate-resistant creeping bentgrass is the first GE turfgrass species being considered for deregulation and, despite strict preventive measures, the transgene escaped and is widespread in plant populations in the area 3 years after the original transgene source was removed. These findings highlight the potential of transgene flow from creeping bentgrass, and question the potential for coexistence of GRCB and non-GE grass seed crops. In our opinion, gene flow from GRCB is a greater challenge for agronomic production than it is an environmental risk. Due to the inherent differences in species biology and the traits conferred by transgenes, it is impossible to generalize the potential of transgene flow and persistence for all GE crops. However, the information presented here should be considered when evaluating the use of genetic engineering technology in outcrossing, wind-pollinated, perennial, small-seeded crops, and when designing risk assessments for the release and deregulation of such GE crops. High diligence is required for traits that confer enhanced fitness or that may represent a threat to the environment.

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Supplementary material

The following supplementary material is available for this article.

Fig. S1. Main irrigation canal in the control area.

Fig. S2. Well vegetated irrigation ditch in the control area.

Fig. S3. Drainage ditch with glyphosate-resistant creeping bentgrass outside the control area.

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