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NEWS AND VIEWS

PERSPECTIVE

Illegal gene flow from transgenic creeping bentgrass: the saga continues

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Ecologists have paid close attention to environmental effects that fitness-enhancing transgenes might have following crop-to-wild gene flow (e.g. Snow *et al.* 2003). For some crops, gene flow also can lead to legal problems, especially when government agencies have not approved transgenic events for unrestricted environmental release. Creeping bentgrass (*Agrostis stolonifera*), a common turfgrass used in golf courses, is the focus of both areas of concern. In 2002, prior to expected deregulation (still pending), The Scotts Company planted creeping bentgrass with transgenic resistance to the herbicide glyphosate, also known as RoundUp®, on 162 ha in a designated control area in central Oregon (Fig. 1). Despite efforts to restrict gene flow, wind-dispersed pollen carried transgenes to florets of local *A. stolonifera* and *A. gigantea* as far as 14 km away, and to sentinel plants placed as far as 21 km away (Watrud *et al.* 2004). Then, in August 2003, a strong wind event moved transgenic seeds from windrows of cut bentgrass into nearby areas. The company's efforts to kill all transgenic survivors in the area failed: feral glyphosate-resistant populations of *A. stolonifera* were found by Reichman *et al.* (2006), and 62% of 585 bentgrass plants had the telltale CP4 EPSPS transgene in 2006 (Zapiola *et al.* 2008; Fig. 2). Now, in this issue, the story gets even more interesting as Zapiola & Mallory-Smith (2012) describe a transgenic, intergeneric hybrid produced on a feral, transgenic creeping bentgrass plant that received pollen from *Polypogon monspeliensis* (rabbitfoot grass). Their finding raises a host of new questions about the prevalence and fitness of intergeneric hybrids, as well as how to evaluate the full extent of gene flow from transgenic crops.

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To date, only a few other cases of spontaneous gene flow from transgenic crops to wild, weedy, or feral populations have been reported (e.g. Hall *et al.* 2000; Warwick *et al.* 2008; Schafer *et al.* 2011), although others are likely to follow when more species of transgenic crops are released near compatible relatives. For example, transgenic rice has been cultivated near weedy rice in the USA and China (where it is still regulated), and genetically engineered traits are expected to spread to conspecific weedy rice populations (Yang *et al.* 2011).

During 2003–2006, Zapiola *et al.* (2008) screened hundreds of *in situ* bentgrass plants and thousands of their progeny to test for glyphosate resistance. A glyphosate-resistant plant found inside the control area in 2005 produced a transgenic seedling that appeared to be a hybrid. Using a combination of nuclear and maternally inherited DNA markers, the authors identified this 'off-type' seedling as an intergeneric hybrid with rabbitfoot grass. The F_1

Fig. 1 Creeping bentgrass and rabbitfoot grass occur along canals and irrigation ditches within the designated control area near Madras, Oregon. Photo courtesy of M. L. Zapiola.

Fig. 2 A feral, glyphosate-resistant creeping bentgrass plant established along a ditch within the control area. Photo courtesy of M. L. Zapiola.

hybrid produced stolons, flowered without vernalization, and produced 14 viable seeds from 90 florets in bagged panicles. The current *Agrostis* study is noteworthy because:

1 This is the first report of spontaneous gene flow resulting in a transgenic, intergeneric hybrid;
2 It is a clear example of the otherwise poorly understood extent of spontaneous hybridization in *Agrostis* and *Polypogon* spp.;
3 In this context, herbicide resistance from a known transgenic event (ASR368) provided a powerful method for tracking and understanding gene flow in the *Agrostis* complex;
4 The study provides valuable information regarding gene flow in a naturalized, non-native, perennial, outcrossing species that is considered to be a weed in some crops and nonagricultural habitats in the USA and elsewhere; and
5 These results question the efficacy of the stringent procedures put in place for preventing unintended gene flow from field trials of regulated transgenic plants.

Public-sector researchers have been very resourceful in taking advantage of this 'natural experiment' in the face of miss-steps and ongoing eradication efforts by The Scotts Company.

Previously, Monsanto Company and Scotts had described the potential for intergeneric hybrids with rabbitfoot grass in a petition to USDA for nonregulated status (Frelich *et al.* 2003), stating that 'on the rare occasions that hybrids have been noted, the hybrids have been sterile (Björkman 1960)'. Low fertility is common in hybrids and is not surprising here given that creeping bentgrass and rabbitfoot grass have different chromosome numbers. However, spontaneous backcrossing and/or loss of extraneous chromosomes has been shown to restore fertility in other crop-wild systems, including intergeneric hybrids between winter wheat (*Triticum aestivum*) and weedy jointed goatgrass (*Aegilops cylindrica*) (e.g. Perez-Jones *et al.* 2010 and references therein). In fact, jointed goatgrass was able to acquire a mutation-derived gene for resistance to imazamox, an imidazolinone herbicide, from wheat in a commercial field in Oregon. *Brassica napus* (canola) and weedy *B. rapa* also have different chromosome numbers but transgenes have been shown to introgress into diploid *B. rapa* following spontaneous hybridization in Canada (Warwick *et al.* 2008). A logical next step for the bentgrass system is to create advanced-generation intergeneric hybrids to study their mating system, ploidy, fitness and competitive ability, and to include *P. monspeliensis* in future risk assessments.

In summary, the saga of Roundup Ready® creeping bentgrass offers valuable lessons about how to prevent the escape of regulated transgenes *via* pollen, seeds and vegetative propagules. Scotts Company was fined $500 000 in 2007 for allowing regulated transgenes to escape, perhaps irretrievably. Meanwhile, in October 2010, the presence of transgenic creeping bentgrass was confirmed in a new area in eastern Oregon, directly across the state border from field trials that were carried out by the Scotts Company in Idaho (Mallory-Smith 2011). Further studies should examine whether the ongoing and accidental spread of glyphosate-resistant perennial grasses represents an environmental and/or economic concern and, if so, how to address it.

References

Björkman SO (1960) Studies in *Agrostis* and related genera. *Symbolae Botanicae Upsalienses XVII*, **1**, 1–114.

Frelich J, Huber S, Nelson E, Stone T (2003) Petition for determination of nonregulated status of Roundup Ready® creeping bentgrass (*Agrostis stolonifera* L.) event ASR368 for determination of nonregulated status. http://www.aphis.usda.gov/brs/aphisdocs/03_10401p.pdf.

Hall L, Topinka K, Huffman J, Davis L, Good A (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple resistant *B. napus* volunteers. *Weed Science*, **48**, 688–694.

Mallory-Smith C (2011) Paper presented at the 64th Annual Meeting of the Western Society of Weed Science, Spokane, WA, USA, March 8.

Perez-Jones A, Martins B, Mallory-Smith CA (2010) Hybridization in a commercial production field between imidazolinone-resistant wheat (*Triticum aestivum*) and *Aegilops cylindrica* results in pollen-mediated gene flow of Imi1. *Weed Science*, **58**, 395–401.

Reichman JR, Waltrud LS, Lee EH *et al.* (2006) Establishment of transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) in nonagronomic habitats. *Molecular Ecology*, **15**, 4243–4255.

Schafer MG, Ross AA, Londo JP, *et al.* (2011) The establishment of genetically engineered canola populations in the US. *Public Library of Science One*, **6**, e25736, DOI: 10.1371.

Snow AA, Pilson D, Rieseberg LH *et al.* (2003) A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecological Applications*, **13**, 279–286.

Warwick SI, Legere A, Simard M-J, James T (2008) Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population *Molecular Ecology*, **17**, 1387–1395.

Watrud LS, Lee EH, Fairbrother A *et al.* (2004) Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. *Proceedings of the National Academy of Science of the USA*, **101**, 14533–14538.

Yang X, Hiu X, Wang F *et al.* (2011a) Transgenes for insect resistance reduce herbivory and enhance fecundity in advanced generations of crop-weed hybrids of rice. *Evolutionary Applications*, **4**, 672–684.

Zapiola ML, Mallory-Smith CA (2012) Crossing the divide: gene flow produces intergeneric hybrid in feral transgenic creeping bentgrass population. *Molecular Ecology*, **21**, 4672–4680.

Zapiola ML, Campbell CK, Butler MD, Mallory-Smith CA (2008) Escape and establishment of transgenic glyphosate-resistant creeping bentgrass *Agrostis stolonifera* in Oregon, USA: a 4-year study. *Journal of Applied Ecology*, **45**, 486–494.

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