Report of the Turfgrasses Working Group

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GENERAL INFORMATION

Over 30 species of grasses are utilized for turf (Huff 1998), while others are important in agriculture as forage crops. The commercial value of this group of plants makes them attractive for improvement through modern genetic engineering techniques (Johnson and Riordan, *in press*). Because of the diversity of species and the consequent differences in biology among them, broad generalizations regarding the ecological effects of pest resistance genes introduced into these crops cannot be made. Rather, questions regarding the potential for pest resistance genes must be directed toward specific cases in which the species and the particular introduced gene are known. In keeping with this approach, particular attention at this meeting was paid to the turfgrasses—in particular creeping bentgrass (*Agrostis palustris* Huds.) and Kentucky bluegrass (*Poa pratensis* L.)—since these are the two grass species that have had transgenic lines tested in the field, and therefore are the species that present the greatest likelihood of being commercialized in the near future.

Major Pests and Diseases

The major pests and diseases that attack turfgrasses are listed in the table below (T. Riordan, pers. comm.). A comprehensive description of turfgrass diseases is published by the American Phytopathological Society (Smiley *et al*. 1992).

Traits Introduced by Breeding

Breeding for disease resistance, greater adaptability to environmental conditions, and turf quality, while maintaining or improving seed yield (in seeded species), have been the main goals of turfgrass breeders. These improvements are typically accomplished through traditional plant breeding, which usually involves the crossing of domesticated genotypes and subsequent selection of cultivars that display the desired trait. Often the genetic control of these traits is not clear and the degree of resistance is not complete (P. Johnson, pers. comm.). Traits that have been introduced by breeding into commercial cultivars include resistance to stem rust and leaf rust (*Puccinia* spp.), brown patch (*Rhizoctonia solani* Kuehn), summer patch (*Magnaporthe poae* Landschoot and Jackson), chinch bugs (*Blissus* spp.), and SAD (Panicum mosaic) virus. In addition, tolerance to heat, salt, and cold has been bred into various turfgrass cultivars.

Traits Introduced by Genetic Engineering

Genetic transformation of turfgrass species is reviewed briefly by Johnson and Riordan (*in press*) as well as by Spangenberg *et al*. (1998). The first trait to be introduced into turfgrasses by genetic engineering was resistance to the herbicide glufosinate in creeping bentgrass. Subsequently, resistance to another herbicide, glyphosate, has also been introduced, as have genes conferring resistance to fungi, viruses, and insects, or tolerance to stresses such as drought,

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salt, and aluminum (references in Johnson and Riordan, *in press*; Information Systems for Biotechnology, 1999).

Weeds of Turfgrasses

With the exception of bermudagrass, turfgrass species are not known to be weeds of other agricultural crops. Among turfgrasses, the most problematic weeds are other species or varieties of turfgrass. In particular, *Poa annua* is an important weedy species (P. Johnson, pers. comm.), and *Eleusine indica* (L.) Gaertn. presents a problem in some areas (J. Neal, pers. comm.).

Degree of Domestication

The turfgrasses varieties used in lawns and golfcourses are an extremely domesticated group compared with their wild progenitor species. The agricultural varieties of creeping bentgrass most likely originated from pastures in northern Europe, while bluegrasses probably came from central Europe (P. Johnson, pers. comm.). They have been selected for their ability to survive close mowing and intense management. Turfgrasses are relatively slow-growing, small in stature, and quickly shaded or out-competed by most plants (Johnson and Riordan, *in press*). In general, the traits selected by breeders have been those that are deleterious to the ability of these species to survive in an unmanaged environment.

Crop Management

Management of turfgrasses is labor intensive, and management practices select for varieties with specialized traits. Soil composition varies among particular areas of golf courses (P. Johnson, pers. comm.); fairways and tees are normally constructed from native soil, but golf course greens are usually constructed with sandy soil mixes (95% sand, 5% peat). Since the water-holding capacity of the greens is low, frequent watering is necessary, especially in warm and dry weather. During warm periods when temperatures on golf greens can exceed 120°F, daily watering is common, and small amounts of additional water are applied during mid-day to cool the plants. Mowing is frequent (6-7 times per week), since the height of the plants is kept at 1/10-1/4". Soil nutrient levels are carefully monitored; nitrogen and potassium levels are maintained at 2-7 lbs/1000 sq. ft./year.

Specialized cultivation practices are also employed (P. Johnson, pers. comm.). Greens, tees, and fairways are “core aerified.” During this procedure, cores of soil measuring 2-4" long, 1/4-3/4" in diameter, and spaced 3/4-1" apart, are pulled from the turf surface. The resulting holes are filled with sand. Many of the new cultivars of creeping bentgrass are frequently mowed vertically; blades that are held perpendicularly to the soil on a rapidly spinning shaft are used to cut stolons and reduce thatch buildup in the turf. This is done regularly, varying from every day on some greens to twice a year on some fairways.

Weed Management

Weeds are controlled by a variety of methods (P. Johnson and J. Neal, pers. comm.). Physical measures to control such weeds as *Poa annua* include hand picking, careful water management, fertility management, cultivation as described in the previous section, and mowing. In addition, growth regulators and herbicides (e.g., “Prograss” [Ethofumesate]) are employed. The careful attention given to weed control places a high value on new transgenic varieties that are herbicide resistant.

WEEDINESS POTENTIAL OF THE CROP

The working group considered evidence that introduction of a pest resistance trait could increase the ability of the crop to become established, persist, or spread. Diseases are clearly a factor in the distribution of turfgrasses as crops. Thus, disease resistances have enabled more extensive planting of turfgrasses within the range in which they are used. Examples of traits that have allowed more extensive planting are resistance to summer patch, Phythium blight (*Pythium* spp.), brown patch, and snow mold. It is important to note that turfgrass spread is due to human planting rather than any inherent ability conferred on these crops to spread on their own as a consequence of disease resistance. In the same way, tolerance to heat, salt, and other environmental stress tolerance have expanded the range in which these crops can be grown.

ROLE OF PESTS IN LIMITING CROP-RELATED WEEDS

To evaluate potential ecological effects of introduced pest resistance genes, the first step is to examine evidence that pests have a significant effect on populations of plant species that are sexually compatible with the crop. The working group was unaware of any evidence that introduction of a disease or pest resistance trait had resulted in the release of turfgrasses or their sexually compatible relatives from any control exerted by those diseases or pests. However, the potential utility of studying endophytes was discussed. Turfgrasses are known to obtain significant benefits from endophytic fungi, which confer greater overall vigor on the plants and therefore might provide greater tolerance to pests or disease. In this respect, evidence obtained from the comparative study of plants with and without endophytes may provide insight into effects of genes that confer resistance to pests, pathogens, or environmental stress. The effect of endophytes might also be useful as a model of the effects of broad pest resistance genes on the fitness and potential weediness of the crop species.

As with the lack of evidence concerning the effect of pest resistance genes on turfgrasses, there was also a lack of knowledge regarding the similarity of pests and pathogens attacking crops and their sexually compatible relatives. It was assumed that the pests affecting the crops also affected sexually compatible species. However, this lack of information was seen as an important gap that needs to be filled.

CONSEQUENCES OF PEST RESISTANCE GENE FLOW

The working group began its consideration of this issue with a discussion on the potential effects of herbicide tolerance. It was concluded that herbicide tolerance would probably not make these crops more invasive. Creeping bentgrass and Kentucky bluegrass possess several traits that render these species ill-adapted for unmanaged situations; hence, the single trait of herbicide resistance was judged to be insufficient to cause these species to become weedy. However, the effect that engineering glyphosate resistance in bentgrass could have on annual bluegrass was raised as a concern. In this case, the effect would not be caused by the transfer of the herbicide resistance trait from bentgrass to annual bluegrass; rather, use of glyphosate on the bentgrass would raise the selection pressure exerted upon annual bluegrass. The emergence of resistance within this species would be accelerated, leading to the consequent loss of glyphosate as a weed management tool in golf course greens. The net result would be reversion to the present situation in which control of annual bluegrass in bentgrass by spraying with glyphosate is not possible. Despite this concern, the potential benefits of glyphosate resistance (reduced use of herbicide, a more environmentally friendly herbicide) was judged to counteract the concern presented.

After further discussion of the consequences of pest resistance, the group concluded that an assessment of the potential effects of pest resistance traits required a case-by-case evaluation. Important considerations in this assessment included the type and mechanism of resistance. In particular, broad spectrum pest resistance was viewed as being of special concern. Therefore, a hypothetical example was considered in which broad spectrum pest resistance was engineered into creeping bentgrass and subsequently transmitted to the sexually compatible species, redtop (*Agrostis gigantea*). For this specific example, it was judged unlikely that pests or pathogens limited populations of bentgrass or redtop. Therefore, even the addition of broad spectrum pest resistance would be unlikely to convert either species to a weed. Additionally, bentgrasses are perennials that do not display many traits seen for typical weeds. Based on these reasons, the addition of pest resistance was seen to be of little concern in the cases of bentgrass and redtop. On the other hand, it was recognized that the conclusion could be different for a species such as buffalograss, which is more likely to have populations controlled by pests or pathogens.

INFORMATION NEEDED FOR RISK ASSESSMENT

The group concluded that we are currently lacking important information for evaluating the

effect of pest resistance genes on the establishment, persistence, and spread of the crop or its sexually compatible relatives. Basic information on the natural history and biology of turfgrasses, as well as weeds in general, was seen as important in evaluating the effect on weediness of an introduced resistance gene in the crop or sexually compatible relatives. Specific areas where information should be obtained or compiled are:

- The life history and invasiveness of the various turfgrass species.
- The geographic range of related species, as well as the cross-compatibility of those species with crop species. Some information on crossing relationships is already known (see for example, Johnson and Riordan, *in press*). However, local variations in genotype and ploidy will result in different rates of transmission of a transgene to sexually compatible relatives.
- The range of pests and pathogens that attack the sexually compatible relatives.
- The factors (including pests and pathogens) that limit populations of sexually compatible relatives.
- The rate of increase of populations of sexually compatible relatives, and the factors that control them.
- A greater understanding of the characteristics of weeds in general. A more thorough study of the characteristics that predispose plants to becoming weeds is needed.

SOURCES OF INFORMATION AND EXPERIMENTAL APPROACHES

The information listed above can be obtained from a number of sources or through experimentation. The committee discussed the following sources and general approaches:

1. <u>Manuals/Literature.</u> Much pertinent information already exists and should be compiled from the literature to provide a useful database for risk assessment. With respect to pathogens infecting sexually compatible species of turfgrasses, information exists from surveys such as those conducted on fungi (Roane and Roane 1994, 1996, 1997) or maize dwarf mosaic virus (Rosenkranz 1981). Such information can also be found on the internet, for example at: http://biology.anu.edu.au/research-groups/MES/vide/refs.htm.

2. <u>Introduction experiments</u>. Introduction of transgenic plants into wild populations of sexually compatible relatives may be a useful approach to consider. Monitoring the ability of various transgenes to confer fitness advantages to plants in these populations would provide information on their potential to cause or enhance weediness.

3. <u>Simulation experiments</u>. Provide a particular genotype with an advantage by artificially increasing the input of seed into an experimental area. This experiment can be conducted with defined genotypes of non-transgenic plants.

4. <u>Experimental crosses</u>. Produce hybrids between selected transgenic crop species and sexually compatible relatives that may have weediness potential. These crosses may then be characterized in experimental plots or greenhouse experiments to assess their weediness.

EXTRAPOLATING FROM SMALL-SCALE FIELD TESTS TO LARGE-SCALE USE

Extrapolation from small to large scale was not seen to present as great a problem in turfgrasses as it may in other crops. In the case of creeping bentgrass, releases will be on a relatively small scale, since golf courses are typically only 100-200 acres, and bentgrasses make up even a smaller proportion of that area (about three acres). Consequently, any information that might be obtained in small scale risk assessment studies could be readily extrapolated to commercial-scale release. The fact that management practices are relatively uniform throughout the range of commercial releases also increases the applicability of data obtained from small scale tests to wide-scale releases. However, certain factors could affect the ability to extrapolate from small scale to large scale:

Region
The region where transgenic turfgrasses are used may affect the applicability of data extrapolated from small to large scale. Regional differences that might affect this include climatic differences and the distribution of sexually compatible relatives.

Scale
Although the original releases of transgenic turfgrasses will be in the commercial market (golf courses), development of transgenic varieties for the homeowner market will involve larger scale releases.

Pollen Spread
As a result of turfgrass crop management and the production of seed for that crop, much of the potential for pollen production and gene flow will be reduced. For the typical end user (golf courses), frequent mowing ensures that plants rarely go to seed. Therefore, transmission of transgenes to sexually compatible relatives should be greatly reduced compared to what would occur if the crop were allowed to flower and produce seed. There is an economic incentive for the producer to prevent cross-pollination during seed production, therefore, isolation of production plots from each other and from sexually compatible wild relatives will also be well controlled, as are production fields of any other crop.

Isolation
However, gene flow might still be frequent and commonplace. Stray plants at edges of fairways or on abandoned golf courses would produce seed, as would plants growing from seeds dropped or scattered during resowing. Though this might not occur on a large scale, the effect might be significant. In production fields where plants are allowed to flower, grass pollen can move several hundred meters or more. Therefore, as with any other crop, complete isolation cannot be assured during seed production.

Effects of Gene Flow
Although there are routes for gene flow between transgenic turfgrasses and their wild relatives, it is unclear whether a transgene would spread once it escapes and what its effect may be if it does spread. These questions can only be answered on a case-by-case basis.

The use of small scale trials to predict performance on a large scale is a standard tool of plant variety development. In plant breeding, there is a long history of extrapolating performance based on small plot trials. In the case of turfgrasses, knowledge of performance is gained through National Turfgrass Evaluation Trials. Although the information obtained from these trials does not usually address the issue of wild relatives becoming weeds, considerable observational data on the weediness potential of the crops themselves could be gathered from these types of trials.

Table 1. Pests And Pathogens Of Turfgrass

Kentucky bluegrass
Ascochyta leaf blight (*Ascochyta* spp.)
Billbug (*Sphenophorus* spp.)
Chinch bugs (*Blissus* spp.)
Curvularia blight (*Curvularia* spp.)
Dollarspot (*Sclerotinia homoecarpa*)
Fall armyworm (*Spodoptera frugiperda*)
Fusarium blight (*Fusarium roseum* and *F. tricinctum*)
Greenbug (*Schizaphis graminum*)
Leafspot (*Drechslera poae*)
Necrotic ringspot (*Leptosphaeria korrae*)
Powdery mildew (*Erysiphe graminis*)
Rust (*Puccinia* spp.)
Sod webworm (Pyralidae)
Stripe smut (*Ustilago striiformis*)
Summer patch (*Magnaporthe poae*)
White grubs (Scarabaeidae)

Creeping bentgrass
Ataenius (*Ataenius spretulus*)
Brownpatch (*Rhizoctonia solani)*
Curvularia blight (*Curvularia* spp.)
Cutworms (Noctuidae)
Dollarspot (*Sclerotinia homoecarpa*)
Fusarium blight (*Fusarium roseum* and *F. tricinctum*)
Gray snowmold (*Typhula* spp.)
Pink snowmold (*Fusarium nivale*)
Pythium (*Pythium* spp.)

St. Augustinegrass
Brownpatch (*Rhizoctonia solani*)
Chinch bugs (*Blissus* spp.)
Curvularia blight (*Curvularia* spp.)
Fire ants
Gray leafspot (*Piricularia grisea*)
Mole crickets (*Scapteriscus* spp.)
Pythium (*Pythium* spp.)
St. Augustine Decline (SAD virus)

Tall fescue
Ascochyta leaf blight (*Ascochyta* spp.)
Ataenius (*Ataenius spretulus*)
Billbug (*Sphenophorus* spp.)
Brownpatch (*Rhizoctonia solani*)
Chinch bugs (*Blissus* spp.)
Curvularia blight (*Curvularia* spp.)
Cutworms (Noctuidae)
Fall armyworm (*Spodoptera frugiperda*)
Fusarium blight (*Fusarium roseum* and *F. tricinctum*)
Greenbug (*Schizaphis graminum*)
Leafspot (*Bipolaris* spp.)
Net blotch (*Helminthosporium* spp.)
Rust (*Puccinia* spp.)
Sod webworm (Pyralidae)
White grubs (Scarabaeidae)

Perennial ryegrass
Ascochyta leaf blight (*Ascochyta* spp.)
Billbug (*Sphenophorus* spp.)
Brownpatch (*Rhizoctonia solani*)
Fusarium blight (*Fusarium roseum* and *F. tricinctum*)
Gray snowmold (*Typhula* spp.)
Pink patch (*Limonomyces roseipellis*)
Pink snowmold (*Fusarium nivale*)
Pythium (*Pythium* spp.)
Red thread (*Laetisaria fuciformis*)
Rust (*Puccinia* spp.)
Sod webworm (Pyralidae)
White grubs (Scarabaeidae)

Bermudagrass
Curvularia blight (*Curvularia* spp.)
Fire ants
Mole crickets (*Scapteriscus* spp.)
Scale (*Odonaspis ruthae*)
Spring dead spot
Stunt Mites (*Aceria neocynodonis*)

References:

Huff DR. 1998. Genetic characterization of open-pollinated turfgrass cultivars. In *Turfgrass biotechnology: Cell and molecular approaches to turfgrass improvement*, eds. Mariam B. Sticklen and Michael P. Kenna, Ch 2, 19-30. Chelsea, Michigan: Ann Arbor Press.

Information Systems for Biotechnology, 1999, http://www.isb.vt.edu.

Johnson PJ and Riordan TP. 1999. A review of issues pertaining to transgenic turfgrasses. *HortScience*, *in press*.

Roane CW and Roane MK. 1997. Graminicolous fungi of Virginia: Fungi associated with genera *Echinochloa* to *Zizania*. *Virginia Journal of Science* 48:11-45.

Roane CW and Roane MK. 1996. Graminicolous fungi of Virginia: Fungi associated with genera *Aegilops* to *Digitaria*. Vir*ginia Journal of Science* 47:197-224.

Roane CW and Roane MK. 1994. Graminicolous fungi of Virginia: Fungi associated with genera cereals. *Virginia Journal of Science* 45:279-296.

Rosenkranz E. 1981. Host range of maize dwarf mosaic virus. In *Virus and virus-like diseases of maize in the United States*, eds. DT Gordon, JD Knoke, and GE Scott, 152-162. Southern Cooperative Series Bulletin 247, June.

Smiley RW, Dernoeden PH, and Clarke BB. 1992. *Compendium of Turfgrass Diseases*. 2d ed. St. Paul, Minnesota: American Phytopathological Society.

Spangenberg G, Wang Z-Y, and Potrykus I. 1998. Biotechnology in Forage and Turf Grass Improvement. *Monographs on Theoretical and Applied Genetics 23*. Berlin: Springer.