Biotechnology-derived, Perennial Turf & Forage Grasses: Criteria for Evaluation

CAST

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Task Force Members

Michael Kenna (Cochair), U.S. Golf Association, Green Section Research, Stillwater, Oklahoma

William K. Hallman (Cochair), Food Policy Institute, Rutgers University, New Brunswick, New Jersey

Carol A. Auer, Department of Plant Science, University of Connecticut, Storrs

Michael D. Casler, USDA–ARS, U.S. Dairy Forage Research Center, Madison, Wisconsin

Andrew Hopkins, Forage Improvement Division, Noble Foundation, Inc., Ardmore, Oklahoma

Keith J. Karnok, Department of Crop and Soil Sciences, University of Georgia, Athens

Carol Mallory-Smith, Department of Crop and Soil Science, Oregon State University, Corvallis

Robert C. Shearman, Department of Agronomy and Horticulture, University of Nebraska, Lincoln

John C. Stier, Department of Horticulture–Extension, University of Wisconsin, Madison

Charles M. Taliaferro, Department of Plant and Soil Sciences, Oklahoma State University, Stillwater

Fred Yelverton, Department of Crop Science, North Carolina State University, Raleigh

Reviewers

Peter Day, Emeritus, Cook College, Rutgers University, New Brunswick, New Jersey

Karen Hokanson, Department of Horticultural Science, University of Minnesota, St. Paul

Peter Jenkins, International Center for Technology Assessment, Washington, D.C.

Kevin B. Jensen, USDA–ARS Forage and Range Research Laboratory, Utah State University, Logan

Marilyn Jordan, The Nature Conservancy, Cold Spring Harbor, New York

Hector Quemada, Crop Technology Consulting, Inc., Kalamazoo, Michigan

John Rooney, Emeritus, University of Oklahoma, Stillwater

Alison A. Snow, Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus

J. Bryan Unruh, Department of Environmental Horticulture, University of Florida, Milton

Workshop Steering Committee

William K. Hallman (Cochair), Food Policy Institute, Rutgers University, New Brunswick, New Jersey

Michael Kenna (Cochair), U.S. Golf Association, Green Section Research, Stillwater, Oklahoma

Keith J. Karnok, Department of Crop and Soil Sciences, University of Georgia, Athens

Virgil Meier, U.S. Department of Agriculture, Animal and Plant Health Inspection Service

Cindy Lynn Richard, Program Coordinator, Council for Agricultural Science and Technology

John Rooney, Emeritus, University of Oklahoma, Stillwater

Brian Steinwand, U.S. Environmental Protection Agency

Dennis Szuhay, U.S. Environmental Protection Agency

John Turner, U.S. Department of Agriculture, Animal and Plant Health Inspection Service

J. Bryan Unruh, Department of Environmental Horticulture, University of Florida, Milton

James White, U.S. Department of Agriculture, Animal and Plant Health Inspection Service

Workshop Contributors

Workshop Sponsors

United States Department of Agriculture–Animal and Plant Health Inspection Service
The Council for Agricultural Science and Technology

Additional Contributors

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Acknowledgments

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The Steering Committee thanks Carol Auer and Brian Unruh for serving as rapporteurs during the workshop. Their careful work helped to make the breakout session discussions more informed and productive. Also, to ensure fair and balanced treatment during the breakout sessions, two professional facilitators led the participatory discussions. Our appreciation to the two facilitators, Warren Flint and Aimee Hill, for a job well done.

And finally, CAST and the Steering Committee thank all those people who participated in the process by submitting their comments on the topics addressed and the structure of the workshop. CAST encouraged members of the public to share facts, thoughts, and questions prior to, during, and for one month after the workshop.

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Foreword

Following a recommendation by the CAST National Concerns Committee, the CAST Board of Directors authorized development of a workshop to address issues regarding biotechnology-derived, perennial turf and forage grasses. An eminent group of experts was chosen as a steering committee to plan and conduct the workshop, which was held in January 2003 in Baltimore, Maryland. After that meeting, a task force cochaired by Drs. Michael Kenna and William Hallman was assembled to prepare this Special Publication. The task force authors attempted to represent in summarized form the contributions made by the diverse group of workshop participants, as well as those who submitted comments before and after the formal meetings.

The task force prepared an initial draft document and revised all subsequent drafts. Invited reviewers read the entire report and provided comments. The CAST Executive Committee and Editorial and Publications Committee reviewed the final draft, and the authors reviewed the proofs. The CAST staff provided editorial and structural suggestions and published the document as an online Special Publication. The task force authors are responsible for the document's scientific content.

On behalf of CAST, we thank the steering committee members, the cochairs, authors, and invited reviewers who gave of their time and expertise to conduct the workshop and prepare the Special Publication as a contribution by the scientific community for public understanding of the issues. We also thank the employers of the scientists, who permitted participation of these individuals at no cost to CAST. CAST thanks all members who made additional contributions to assist in the preparation of this document. The members of CAST deserve special recognition because their unrestricted contributions in support of CAST financed the preparation and online presentation of this Special Publication.

Dale M. Maronek
President

Teresa A. Gruber
Executive Vice President

Linda M. Chimenti
Managing Scientific Editor

Interpretive Summary

By using conventional methods, grass breeders have created hybrids with superior tolerance to extremes in temperature, light, and salinity and environmental stresses such as drought. Certain grasses also have been bred for improved vegetative spreading ability, seed yield, and traffic tolerance. A few of the goals of those breeding perennial grasses have included improved vegetative spreading ability, seed yield, and traffic tolerance, as well as plants that grow well in extreme conditions, resist pests and diseases, provide better nutrition for animals, and require less water, fertilizers, and pesticides. Such hybrids would help protect scarce natural resources and wildlife habitat and would enable more economical management practices for turf and forage grasses. Breeders increasingly are turning to biotechnology to achieve these goals because of its potential to improve the speed of breeding to allow specific traits to be modified within a species and to provide opportunities for genetic modifications that are difficult or impossible to achieve using conventional methods. Because of the use of biotechnology to create such hybrids, however, U.S. government approval is required during hybrid development and before the product is sold to consumers.

Deregulation of Biotechnology-derived Perennial Grasses

Deregulation would permit the unconfined release of a biotechnology-derived (BD) grass species and allow them to be cultivated widely with few or no restrictions. Before granting deregulation of BD plants, the U.S. Department of Agriculture (USDA)–Animal and Plant Health Inspection Service (APHIS) conducts a prospective risk analysis, a process that evaluates the potential risks of a plant or gene not yet released into the environment. Key to this assessment is an evaluation of whether the new BD plant is likely to pose a greater plant pest risk than the unmodified organism from which it was derived. Of particular importance is whether there are biologically significant changes in the plant's form or structures, or in its ability to reproduce or survive.

Although APHIS has an established set of criteria to guide evaluation of *all* BD plants before deregulation, BD perennial turf and forage grasses are a unique group of plants with plant parts, growth, and reproductive habits that differ significantly from those crops that already have passed through the deregulation process. Careful attention to the criteria used for deregulation of BD perennial grasses is needed because of the following:

- the diverse type, numbers, and widespread distribution of grasses throughout U.S. urban, agricultural, and natural areas;
- the many species that reproduce through wind-blown pollination, which presents the possibility of gene flow between the BD species and other related grasses in the wild or in areas of conventional seed production; and
- genes that cause a plant to become more fit than its non-BD varieties or wild relatives conceivably could enhance the plant's potential to become an invasive weed. Factors that breeders would like to add to existing varieties that may enhance fitness also are traits evaluated by the USDA in their risk assessment of BD plants:
  - superior tolerance to moisture, light, wind, and temperature extremes;
  - enhanced seed production; and/or
  - increased vegetative spread.

Workshop

With partial support from APHIS, the Council for Agricultural Science and Technology (CAST) hosted a gathering of more than 100 scientists, regulators, and representatives of industry and nonprofit organizations from throughout the United States. The objectives of the workshop were to provide a forum for discussion of the current status of BD perennial turf and forage grasses, and to initiate a dialogue on the possible criteria that could be used to determine the environmental safety and potential risks and benefits of these grasses.

Establishing Criteria for Evaluation

Most of the turfgrasses and certain of the forage grasses commonly grown in the United States originated on other continents and were introduced after European colonization. These grasses form a group of genetically and physically diverse plant species that evolved as components of natural grasslands under varied environmental conditions in different parts of the world. As a result, there are significant differences among grass species, including

- physiology,
- rates and mechanisms of growth and reproduction (both sexual and asexual),
- tolerance and adaptability to extreme conditions,
- current and potential geographical distributions of both seed production and cultivation, and
- intended uses.

These differences make a *single* set of criteria for deregulation of all perennial turf and forage grasses impractical. In response, the workshop participants identified three important concepts that should support regulatory decision making concerning the ecological risk assessments central to the process for deregulating BD grasses.

1. The Principle of Familiarity Should Be Used in Helping to Understand Potential Risks

In assessing the potential ecological risks that might be posed by introducing novel traits into BD turf and forage grasses, it should be recognized that much already may be known about

- the nonmodified species from which it was derived,
- the trait that has been introduced,
- the ecosystem in which the plant will be used, and
- the interactions among these.

Although a high degree of *familiarity* does not guarantee safety, high levels of existing knowledge and experience with the non-BD variety allow greater confidence that unintended consequences of a novel trait are unlikely. This permits risk assessors to focus on confirming that the BD plant is the same biologically as the non-BD plant from which it was derived, other than for the intended change. Once this has been established, evaluators can consider the impacts of the intended change.

2. Credible, Transparent Scientific Information Is Needed to Support Decision Making

The importance of credible, transparent scientific knowledge derived from well-designed and adequately replicated experiments was stressed, including the following considerations:

- Because ecological systems are complex, experiments over a range of spatial and temporal scales may be needed to evaluate the potential risks of BD grasses properly.
- Assertions about the presence or absence of adverse ecological effects must be placed in the context of a scientific hypothesis that can be tested with a reasonable amount of time and resources.
- All experiments must be designed effectively and rigorously by using accepted statistical protocols.
- Nonsignificant results should be accompanied by an analysis of statistical power.
- Data should be available to the public and preferably subject to peer review.
- Before information available in nonrefereed publications or reports is accepted, it should undergo an independent scientific review or verification to determine the credibility of the results and conclusions.

3. Evaluations of Biotechnology-derived Grasses Should Occur on a Case-by-Case Basis Using Science-Based Assessment Criteria with a "Tiered" Approach

A case-by-case approach consistent with USDA guidelines should take into consideration

- the specific biology of the BD grass being assessed,
- the specific BD trait that has been introduced,
- potential effects on managed and natural ecosystems in which the BD plant will be grown, and
- other hazards that can be predicted.

Such an assessment typically would include information concerning

- growth and life span,
- vegetative vigor,
- flower production,
- pollination mechanisms and pollen movement,
- seed production, dispersal, yield, and dormancy,
- seedling recruitment and survival,
- overwintering,
- persistence in the soil,
- outcrossing frequency,

- asexual reproduction,
- symbionts, and
- stress adaptations.

The transgene should be described with regard to

- the complete DNA sequence inserted,
- the stability of the transgene,
- inheritance through sexual reproduction, and
- the pattern of gene expression throughout the plant.

In addition, the effect of the transgene on plant growth, reproduction, ability to survive in managed and natural landscapes, stress responses, potential effects on nontarget organisms, and gene flow to sexually compatible plant species should be described for the risk assessment. And consistent with the National Environmental Policy Act, evaluations also should consider potential environmental effects on children, minorities, and low-income populations (including farm workers) and any significant environmental impacts abroad that might result from the deregulation of a BD grass.

To collect and evaluate scientific information about the potential risks of BD grasses, workshop participants endorsed the concept of a "tiered approach," starting with existing knowledge and the concept of familiarity, then moving to higher levels of analysis requiring more complex experiments. If the potential for undesirable ecological effects cannot be eliminated in a lower assessment tier, the next higher tier of experiments and analysis should be conducted on the BD plant. Properly designed and implemented, tiered experiments could provide a systematic and efficient approach to answering questions about environmental risks.

1 Introduction

The Nobel Prize-winning chemist Robert F. Curl of Rice University spoke for many of his colleagues in science when he proclaimed "the twentieth century was the century of physics and chemistry. But it is clear that the next century will be the century of biology." Plant scientists are at the forefront of introducing *biotechnology*,[1] or biotech, into perennial turf and forage grasses, nature's carpet that protects the soil, provides vast amounts of food and habitat for livestock and wildlife, and graces urban areas with color and space to enjoy recreation. This initial effort, along with conventional plant breeding improvements in turf and forage grasses, will enable scientists to decrease water, fertilizer, and pesticide use in the long term.

Appropriate biotechnology may help provide a means for major innovations to the annual, multibillion-dollar turf and forage grass industries. Approximately 350 million hectares (ha) (864 million acres [a.]) of grasses include pasture, grassland, and rangeland that support beef, dairy, sheep, goats, swine, horses, poultry, and wildlife (e.g., deer, songbirds, wildfowl). Turfgrass acreage includes the lawns surrounding more than 70 million detached homes, approximately 17,000 golf courses, more than approximately 121,000 ha (300,000 a.) of sod production, and more than 700,000 sports fields in the United States. Most turf and forage grasses used in North America are not native species, however, and their widespread use and naturalization have impacted natural areas in certain instances. Careful attention to the field testing and deregulation of biotechnology-derived (BD) perennial grasses is needed because of the diverse number and widespread distribution of grasses throughout U.S. urban, agricultural, and natural areas.

If deemed safe for use and commercialization by U.S. regulatory agencies, BD perennial grasses may provide significant environmental and economic benefits. Scientists are seeking solutions to protect scarce water resources and wildlife habitat, to decrease fertilizer and pesticide pollution, and to provide economical management practices for turf and forage grasses.

This publication summarizes a 2-day workshop on the state-of-the-science of BD perennial turf and forage grasses. The goal of the workshop was to provide a forum for discussion of the current status of BD perennial turf and forage grasses and to initiate a dialogue on the possible criteria used to determine the environmental safety and potential risks and benefits of these grasses relative to those derived using traditional breeding approaches. Supported in part by the U.S. Department of Agriculture (USDA)–Animal and Plant Health Inspection Service (APHIS), the Council for Agricultural Science and Technology, a nonprofit consortium of food and agricultural scientists, hosted this gathering of more than 100 scientists, regulators, and representatives of industry and nonprofit organizations from throughout the United States.

There were multiple opportunities throughout the meeting to provide comments, including two public comment sessions and three breakout sessions to facilitate discussion of key questions regarding the evaluation of BD perennial grasses. In each breakout session, the attendees were asked to provide "big picture" issues that should be considered if BD perennial grasses are deregulated. These were open and serious discussions held by a diverse group of stakeholders about the importance of perennial grasses in native, agricultural, and urban settings, as well as the environmental consequences of introducing new BD grasses. Members of the public also were encouraged to submit data, background information, and other comments through written submissions to CAST. The independent submissions can be found in Appendix B. Several opportunities to contribute to the final product were provided for individuals and groups interested in the issues surrounding BD perennial grasses, regardless of attendance at the workshop.

The authors of this publication attempted not only to capture the respective expertise and contributions made by the diverse group of workshop participants and those who submitted comments before and after the formal meetings, but also to provide summaries of a vast amount of scientific information on which

[1]Italicized terms (other than scientific names) are defined in Appendix F: Glossary.

to base future dialogue on this important subject. General policy recommendations and research needs for the development and release of BD perennial grasses are summarized in Chapter 2, "Principles Supporting Ecological Risk Assessment and Regulatory Decision Making."

Chapter 3, "Background Information on Perennial Grasses," overviews turf and forage grasses, including the management, biology, and breeding of perennial grasses. It also explores potential solutions that BD perennial grasses may provide to existing problems and discusses potential weed problems associated with these grasses.

Chapter 4, "Gene Migration and Weed Management of Biotechnology-derived Perennial Grasses," focuses on two major concerns: (1) whether inserted genes will migrate into related species, and (2) how BD grasses will form hybrids with related species and become weed problems.

An important function of this publication is to make information available to government agencies responsible for determining whether or not BD plants should be deregulated. Chapter 5, "Criteria for Evaluating Biotechnology-derived Perennial Grasses," addresses the unconfined release of BD grasses, examining it in the framework of three major categories: seed, vegetative, and flowering characteristics. An extensive list of questions was developed and discussed by the invited speakers and meeting attendees during the workshop's breakout or discussion sessions. A summary of the key issues for each of the three categories is provided in this chapter.

In Chapter 6, "Questions and Answers: A Summary of Workshop Responses and Public Comments," the specific questions posed by the USDA–APHIS concerning the unconfined release of BD perennial grasses are addressed. Comments made at the workshop, subsequent public comments, and author summaries were reviewed in order to provide the answers to the USDA–APHIS list of questions. There is a wide variety of opinions and concerns found throughout the entire document, appendices, and Internet website links; however, it was impossible to reiterate this diverse discussion within Chapter 6 in order to provide succinct guidelines for the USDA–APHIS.

Finally, this publication also includes the following appendices: (A) Web Resources, (B) Link to Public Comments, (C) Participant List, (D) Workshop Agenda, (E) Abbreviations and Acronyms, and (F) Glossary.

2 Principles Supporting Ecological Risk Assessment and Regulatory Decision Making

"What are the principles and methods supporting ecological risk assessment and regulatory decision making for biotechnology-derived (BD) turf and forage grasses?" "What scientific knowledge is needed for credible and rigorous risk assessments?" "Who has a stake in the development of BD grasses, and how can their concerns be addressed?" These are just a few of the questions raised by representatives of the green industry, seed companies, *biotechnology*, or biotech companies, the government, environmental groups, and others during the 2-day workshop. This chapter summarizes certain "big picture" issues discussed during the workshop.

How Biotechnology-derived Grasses Are Evaluated by the U.S. Government Before Receiving Regulatory Clearance

The regulation of all BD plants occurs within an established process for all agricultural biotechnology products called the "Coordinated Framework for Regulation of Biotechnology Products" (U.S. Congress 1986). The Coordinated Framework divides regulatory responsibility and *risk assessments* for BD plants among three agencies: The U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA), and the U.S. Environmental Protection Agency (EPA). The Coordinated Framework and regulatory process for deregulation of BD plants has been described in various government and nongovernmental publications (Belson 2000; CAST 2001; CEQ/OSTP 2001; NAS–NRC 2000). The USDA and other agencies are involved in prospective risk analysis, a process that evaluates the potential future risks of a *stressor* (plant or gene) not yet released into the environment (Nickson and McKee 2002). In a complex process that has operated for over a decade, U.S. laws, policies, risk assessments, and scientific knowledge enable government decision making about whether to permit the widespread use and commercialization of any BD plant.

Under the Plant Pest Act, the USDA–Animal and Plant Health Inspection Service (APHIS) assesses whether the new BD plant is any more likely to pose a greater plant pest risk than the unmodified organism from which it was derived. The APHIS assesses whether the transformed plant is the same as the nontransformed plant and therefore familiar, other than the intended change because of the expression of the *transgene*. Of particular importance is whether there are observable characteristics that would indicate a biologically significant change(s) in the BD plant's morphology and reproductive or survival biology.

The APHIS regulations for BD plants are based on the Plant Protection Act. The APHIS conducts an evaluation of the plant pest and environmental risks that could occur before granting deregulation of BD plants, allowing for cultivation without confinement conditions specified under those regulations. The APHIS regulations (7 CFR 340.6) list the types of data and information that must be provided to demonstrate that the BD plant is unlikely to pose a greater plant pest risk (risks to plants or plant health) than the nonmodified organism from which it was derived. These are interpreted to include, but are not limited to the following:

1. Plant pest risk characteristics (i.e., ability to cause a plant disease, disease symptoms, damage, or injury);
2. Increased disease and pest susceptibilities;
3. Expression of the gene product, new enzymes, changes to plant metabolism that directly or indirectly effect plant health;
4. Weediness or *invasiveness* of the regulated article;
5. Impact on the weediness or invasiveness of sexually compatible relatives;
6. Effects on agricultural or cultivation practices (including organic farming) that adversely effect plant growth and sustainable agriculture;
7. Effects on nontarget organisms, especially threatened and endangered species;
8. Effects on other agricultural products;
9. Indirect plant pest effects (disease, damage, or injury) on other agricultural products; and
10. Transfer of genetic information to organisms with which it cannot interbreed (if applicable).

Any other information that the APHIS believes to be relevant to a determination and any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism also must be included.

Depending on the BD plant under consideration, the developer may seek additional review and clearance by the EPA and the FDA before commercializing the new BD plant. The EPA is responsible for assessing the environmental and nontarget safety of a pesticide produced by a BD plant and to establish food or feed tolerance levels. If the BD plant enables the application of a pesticide that otherwise would not be applied to the conventional counterpart, such as a herbicide, the EPA must approve the new use of the pesticide. The FDA assesses whether the BD plant is compositionally and nutritionally equivalent to its conventional counterpart and therefore equally safe to be consumed as a food or animal feed.

The focus of each agency differs, however, with regard to the data and information required to perform an ecological risk assessment. The workshop identified three important concepts that should support regulatory decision making for BD grasses: (1) evaluation on a case-by-case basis; (2) the principle of familiarity in understanding risk; and (3) the importance of credible, transparent scientific information for decision making.

Evaluation on a Case-by-Case Basis

The first fundamental concept supporting the regulatory framework is that the safety of each BD plant is determined on a case-by-case basis using science-based assessment criteria (CAST 2001; Hokanson et al. 1999; USDA 2002). The case-by-case approach does not consider explicitly the process by which the plant was made (*DNA technology*), but focuses on crop biology, the specific BD trait (transgene and *molecular construct*), potential effects on managed and natural ecosystems in which the BD plant will be grown, and other hazards that can be predicted.

For example, in the case-by-case approach to ecological risk assessments used by the USDA, crop biology is examined using specific guidelines (USDA 2002). A study of BD grass biology would include patterns of growth, life span, vegetative vigor, flower production, pollination mechanisms, pollen movement, seed production, seed dispersal, seed yield, seed dormancy, seedling recruitment and survival, overwintering, persistence in the soil (seed banks), outcrossing frequency, asexual reproduction, *symbionts*, and stress adaptations. The transgene must be described with regard to the complete DNA sequence inserted, stability of the transgene, inheritance through sexual reproduction, and pattern of gene expression throughout the plant. The effect of the transgene on plant growth, reproduction, ability to survive in managed and natural landscapes, stress responses, potential effects on nontarget organisms, and *gene flow* to sexually compatible plant species must be described for the risk assessment.

In addition, because the APHIS typically conducts an environmental assessment according to the National Environmental Policy Act, which also addresses Executive Orders 13045, 12898, and 12114, additional consideration is given to any environmental effects on children, minorities, and low-income populations (including farm workers), as well as any significant environmental impacts abroad that might result from a determination of nonregulated status.

Defining Benefits

Perennial grasses first can be divided into turf and forage, both of which play significant roles in North America. Grasslands include forage, pasture, and range grasses, and together they comprise the largest number of acres for any crop in the United States. The forage, grassland, and range resources of the United States cover about 55% of the land area of the United States. These sustainable resources are found in all 50 states (AFGC 2001) and provide valuable benefits to all Americans in many areas, including:

- Food and clothing from plant and animal products, including meat, milk, and wool;
- Abundant wildlife habitats and aesthetically pleasing landscapes for recreation, enjoyment, and appreciation;
- An alternative source of energy and industrial raw materials; and
- Environmental protection for soil, water, and air.

The economic value of these benefits is very significant. For example, the forage-livestock industry contributes more than $60 billion in farm sales annually, and the $11 billion hay crop is the third most valuable crop after corn and soybeans (AFGC 2001). Equally important, although difficult to quantify in dollars, are the environmental, aesthetic, and recreational benefits, which provide an invaluable public benefit. When grassland agriculture is practiced, soil organic matter is renewed, soil erosion prevented, gully formation arrested, and soil tilth improved (Heath, Metcalfe, and Barnes 1973). Through proper management of grasslands, soil conservation be-

comes an opportunity rather than a problem in agricultural areas.

Turf is used widely in urban areas for lawns, recreational areas, and roadsides. The complexity and comprehensiveness of turf benefits to our quality-of-life were quantitatively documented recently (Beard and Green 1994). Beyond the aesthetic and recreational aspects of turf, the most important contribution is its functional role protecting topsoil from water and wind erosion, storm water retention, and decreasing surface water runoff (Beard 1973; Beard and Green 1994; Linde et al. 1995; Shiflet and Darby 1985). In Table 2.1, the benefits of turfgrass are summarized for functional, recreational, and aesthetic components. Perennial grasses are an economically and environmentally significant set of species in our urban and agricultural landscapes. When discussing the potential risks of BD perennial grasses, it is important to weigh these benefits and assess how conventional or biotechnological improvements will help them make a positive contribution to our quality-of-life.

Despite the benefits of perennial grasses, several concerns have been raised by the general public, as well as by those who use these grasses for forage and turf purposes. These issues include conserving natural resources, minimizing any negative environmental impacts associated with utilization of forage and turf, and improving productivity and economic profitability.

Conservation of Natural Resources

Perennial grasses used in urban settings often require additional irrigation in dry periods. People became concerned about how much water turfgrasses use during a series of widespread droughts in the late 1970s and 1980s. Severe drought in California and other Western states has resulted in extreme water-use restrictions in hundreds of communities, affecting irrigation of lawns, parks, sports fields, and golf courses, due in part to their visibility and because they were considered nonessential uses of water. Irrigation of perennial grasses used for forage in the United States occurs generally only in the Western states. In these areas, interest in more efficient pasture irrigation has increased because of water limitations and drought (Jensen, Asay, and Waldron 2001; Waldron, Asay, and Jensen 2002). The development of drought-tolerant, perennial grasses would help maintain adequate forage yields or turfgrasses that need less irrigation while continuing to provide valuable buffering in urban and agricultural watersheds.

Table 2.1. Functional, recreational, and aesthetic benefits of turfgrass (Adapted from Beard and Green 1994)

Functional	Recreational	Aesthetic
• Soil erosion • Dust prevention • Heat dissipation • Noise abatement • Glare reduction • Air pollution control • Nuisance animal reduction	• Low-cost surfaces • Physical health • Mental health • Safety • Spectator entertainment	• Beauty • Quality of life • Mental health • Social harmony • Community pride • Increased property values • Complements trees and shrubs in landscapes

Minimization of Negative Environmental Impacts

If managed properly, perennial turfgrasses use applied fertilizers efficiently (Kenna and Snow 2000). Nutrients such as nitrogen and phosphorus also are used efficiently in grazing systems that include perennial grasses, although improvements would be helpful in addressing water contamination concerns (Correll 1996). During the rapid suburbanization of several areas in the United States during the 1980s and 1990s, the public asked for an assessment of the environmental impact of turfgrasses. Concerns about the misuse of pesticides and fertilizers on suburban lawns also were used as political arguments in efforts to halt the development of new housing and commercial real estate. This public concern arose throughout the United States in spite of the demand for recreational sites and green spaces such as parks, sports fields, and golf courses.

The effect of pesticides and fertilizers on water supplies is an important issue (Kenna and Snow 2000). Transport (Cisar and Snyder 1996) and exposure levels (Murphy, Cooper, and Clark 1996) after application of pesticides to turf have been documented to be minimal. But the potential for water pollution to impact wildlife exists under certain conditions (Smith and Bridges 1996), including if pesticides and fertilizers are not applied with care. Turfgrasses that use fewer pesticides and fertilizers are being developed to address these concerns through conventional breeding and biotechnology.

Improved Productivity and Economic Profitability

Only a portion of the forage consumed by an animal is digested. Small increases in digestibility of forages, as a result of traditional plant-breeding

methods, have led to great improvements in animal growth and animal weight gains per acre (Casler and Vogel 1999). These weight gains can translate into improved returns for livestock producers (Vogel, Gorz, and Haskins 1989).

Defining Risk and Risk Assessment

When discussing risk and risk assessment with regard to BD plants, and, by extension, perennial grasses, it is important to have a working definition of the two terms. Risk typically is defined by the formula: risk = hazard x exposure. In relation to BD plants, the *hazard* represents the severity of an unwanted environmental change resulting from the release of the BD plant, whereas *exposure* represents the probability that the hazard will occur (Wilkinson, Sweet, and Poppy 2003). Risk assessment is the process of identifying and characterizing precisely the hazard and quantifying the probability that it will occur, with the former (hazard identification) being paramount to this process (NAS 1996; Wilkinson, Sweet, and Poppy 2003). Therefore, it is critical to focus on identified hazards and risks supported by facts. Speculation without facts is not risk assessment and tends to favor the obvious or dramatic rather than those hazards likely to have large-scale environmental consequences (Hokanson et al. 1999; Wilkinson, Sweet, and Poppy 2003).

The Principle of Familiarity in Understanding Risk

The second important concept, that of *familiarity*, has been established as one of the basic principles for the evaluation of BD plants in the United States and internationally (FAO/WHO 2001; Hokanson et al. 1999; Madsen et al. 2002). Familiarity suggests that the more familiar we are with something, the more likely we are to understand, predict, and manage potential risks (FAO/WHO 2001). In BD crops, familiarity encompasses the existing knowledge and experience with a specific crop plant, the BD trait or *phenotype*, the ecosystem in which the plant will be used, and interactions among these elements. The EPA, FDA, and APHIS require information on all observable characteristics that would indicate any biologically significant change in plant morphology and reproductive or survival biology of the BD plant. The purpose is to confirm that the BD plant is the same as non-BD plants, and therefore familiar, other than the intended change because of expression of the transgene. Once this is established, then the APHIS can consider the impacts of the intended change.

Familiarity is not synonymous with safety, but a high degree of familiarity suggests that there will be enough information to judge safety or manage risks (FAO/WHO 2001; Hokanson et al. 1999; Madsen et al. 2002). A lack of familiarity provides an even stronger argument that risks must be studied on a case-by-case basis and in a stepwise manner (FAO/WHO 2001). In certain instances, a low degree of familiarity can be balanced by well-designed experiments or management practices (FAO/WHO 2001).

The Importance of Credible, Transparent Scientific Information for Decision Making

A third fundamental concept broadly supported at the workshop was the importance of credible, transparent scientific knowledge derived from well-designed, adequately replicated experiments. Because ecological systems are complex, experiments at a range of spatial and temporal scales may be needed. But assertions about the absence of adverse ecological effects must be placed in the context of a scientific hypothesis that can be tested with a reasonable amount of time and resources (Nickson and McKee 2002). All experiments must be designed effectively and rigorously using accepted statistical protocols, and nonsignificant results should be accompanied by an analysis of statistical power (Marvier 2002). Data should be available to the public and subject to peer review. The assessment endpoints, defined in the problem formulation phase of the environmental risk assessment, must be measurable plant characteristics. In this respect, the traits of weediness and invasiveness have been criticized because (1) they lack predictive powers, (2) there is no consensus on the criteria for evaluation, and (3) certain desirable traits in a BD grass may overlap weediness characteristics (Kareiva, Parker, and Pascual 1996; Nickson and McKee 2002; NAS–NRC 2002). Despite these difficulties, it is essential that the ability of a BD grass to become a weed in natural areas be considered in the ecological risk assessment. Several different sets of criteria to rank invasive potential have been developed and could be used to assess the invasive potential of BD grasses (Hancock 2003). In fact, it has been suggested that the ability of BD grasses to become weeds deserves special attention.

For collection of scientific information, workshop participants generally promoted the concept of a tiered approach to experiments. One study has described three levels of tiered assessments for BD plants, starting with existing knowledge and the con-

cept of familiarity (Tier 1), and then moving to higher levels of analysis requiring more complex experiments in Tiers 2 and 3 (Nickson and McKee 2002). If the potential for undesirable ecological effects cannot be eliminated in a lower assessment tier, the next higher tier of experiments and analysis would be conducted on the BD plant. Properly designed and implemented, tiered experiments could provide a systematic and efficient approach to answering questions about environmental risks.

Workshop discussions also touched on the importance of peer review and publication of information in scientific journals. Transparent, credible, and rigorous scientific information is essential for government officials who must explain regulatory decisions and build public confidence in biotechnology. Before information from nonrefereed publications or reports is accepted it should undergo an independent scientific review or verification to determine the credibility of the results and conclusions.

Application of the Concept of Familiarity to Biotechnology-derived Grasses

Perennial grasses have been used in urban and agricultural landscapes for hundreds of years without causing harm to animals or humans. A body of "expert knowledge" about grass biology, agronomic characteristics, and management practices resides with scientists and their research programs within different institutions (e.g., state universities, the USDA, seed companies, industry associations). Familiarity with BD traits varies, but the most familiar traits include resistance to pests or tolerance to heat and drought introduced into grasses through traditional breeding. Familiarity also would include traits that have been used in other BD plants.

For example, plant breeders have introduced disease and insect resistance as well as tolerance to heat and drought into grasses through traditional breeding. Experience with BD traits in grasses may vary among scientists; however, there is general knowledge of the application and safety of BD plants such as herbicide- and/or insect-tolerant canola, corn, cotton, and soybeans (CAST 2001). This experience provides tools for turf and forage grass scientists to perform or contribute to the performance of ecological risk assessments and the development of risk management plans for BD grasses.

Most stakeholders support the case-by-case approach and the concept of familiarity; however, the details must be developed for each individual perennial grass species. There were several significant questions and concerns raised at the workshop. For example, which ecological risk assessment criteria apply to BD grasses? What data are needed to support safety claims? How should small-scale greenhouse or field trials be designed and conducted? How should changes in weediness or other traits be measured? Can small-scale experiments or models predict what will happen after deregulation and large-scale use? Will gene flow from BD grasses produce negative environmental impacts? What is the value of expert opinion compared with experimental data? Are there scientific methods to test risk assessment endpoints, such as changes in weediness or gene flow impacts? Furthermore, because plant genetics and the environment interact to create the plant phenotype, how can experiments be designed to predict complex interactions and unintended effects in different ecosystems? How can development of certain legitimate breeding goals be separated from development of traits equated with weediness?

Along with the established criteria to characterize and evaluate BD plant phenotypes (USDA 2002), certain workshop participants suggested that guidelines include testing BD traits in multiple genetic backgrounds (grass varieties, native and naturalized related species) and different environmental conditions found within the United States (winter and summer temperatures, patterns of rainfall, soil conditions, and other variables) to decrease the possibility of unexpected hazards. The ability of BD grass to compete with other plants in natural landscapes should be considered, as well as the possibility of transferring traits (e.g., tolerance to drought, salinity, disease, insects, or herbicides) that allow related species to become undesirable weeds in natural areas.

Limitations of the Case-by-Case Approach, the Concept of Familiarity, and Ecological Risk Assessment of Biotechnology-derived Grasses

As a BD grass moves away from a reference baseline of traditional grass cultivars, the concept of familiarity is less useful as a guideline for risk assessment. Examples of decreased familiarity include the

introduction of a grass species not previously used as a turf or forage, production in new areas, and insertion of BD traits not previously used in other crops.

The process of gene flow presents a challenge for science and regulation, and it received intense discussion at the workshop. Gene flow, the movement of genes between populations of plants through pollen, seeds, or living plants (Hartl and Clark 1997), is a natural process and an important component of speciation (Ellstrand, Prentice, and Hancock 1999). Although crops and wild plants always have exchanged genes, the development of *transgenic* plants has raised considerable debate and stimulated new research because gene flow could allow the movement of novel BD traits into natural or managed ecosystems (Conner, Glare, and Nap 2003; Ellstrand 2000; Ellstrand, Prentice, and Hancock 1999; Snow 2002; Wilkinson 2002; Wipff and Fricker 2001). Prospective risk assessments by federal agencies must determine the level of risk for gene flow from BD grasses and environmental impacts in agricultural systems, home lawns, managed landscapes, and/or natural ecosystems. Workshop participants asked if there is sufficient scientific knowledge to support risk assessment and regulatory decisions.

The first step in a systematic approach is to ask if transgenes could move from BD grasses to other grasses (Conner, Glare, and Nap 2003; Wilkinson 2002). A comparison of certain plant attributes important to ecological risk assessment and gene flow is shown in Table 2.2 using two annual crops (canola and maize) and two perennial plants (creeping bentgrass and hybrid cottonwood trees). Today, BD cotton, canola, and maize and a number of other crops are deregulated in the United States (USDA 2003). To date, virus-resistant papaya planted in certain areas of Hawaii and cotton (typically grown as an annual) are the only BD perennial species to be deregulated. In creeping bentgrass, which has an outcrossing breeding system and wind-blown pollen, research has shown that transgenes could move into nontransgenic grasses in the same species, *feral* (wild) populations of the same species, sexually compatible naturalized species, and/or sexually compat-

Table 2.2. Comparison of plant attributes important to ecological risk assessment for canola (*Brassica napus*), maize (*Zea mays*), creeping bentgrass (*Agrostis stolonifera*), and hybrid cottonwood tree (*Populus trichocarpa x P. deltoides*). Canola and maize are annual row crops, whereas creeping bentgrass and hybrid cottonwood are perennial plants (Adapted from Ellstrand, Prentice, and Hancock 1999; James et al. 1998; Jónsdóttir 1991; Wipff and Fricker 2000, 2001)

Attribute	Canola	Maize	Creeping bentgrass	Hybrid cottonwood
Extent of domestication (genetic distance to wild species)	Moderate	Moderate to high	Low	Low
Geographic origin of wild parent	India, Mediterranean	Mexico, Central America	North America, Europe, N. Asia	North America
Mode of pollination	Insect	Wind	Wind	Wind
Breeding system	Self- and outcrossing	Outcrossing	Outcrossing	Dioecious, outcrossing
Possibility for hybridization with native or naturalized species in the United States	Yes	No	Yes	Yes
Reported as weed in natural landscapes in the United States	No	No	Yes	No
Life cycle and time to flowering	Annual, ~ 45 days	Annual, ~ 63–95 days	Perennial, ~1 year	Perennial, 4–10 years
Persistence of seed in soil	>1 year	>1 year	1–2 years	<2 weeks
Potential for seed dispersal	Moderate	Low	Low	High
Vegetative persistence	No	No	Yes	Yes
Vegetative propagation	No	No	Yes	Yes

ible native grasses (Belanger et al. 2003; Christoffer 2003; Wilkinson 2002; Wipff and Fricker 2000, 2001). In addition, creeping bentgrass seeds or vegetative plant parts (*stolons*) could carry transgenes away from the original planting location.

Given that gene flow is a natural process in grasses and other plant species, it is necessary to determine the likelihood of moving the transgene from one species to another (*introgression*) and ecological effects of the transgene into a population. Difficulties arise in this task because there are many stages to introgression, and the combination of crop species, transgene, and geographic location plays a critical role (Conner, Glare, and Nap 2003; Ellstrand 2000; Ellstrand, Prentice, and Hancock 1999; Snow 2002; Wilkinson 2002). Wilkinson (2002) has described a structured approach that can be applied to studying each stage of the gene flow process. There is concern, however, about limited baseline data and a lack of interdisciplinary research on the effects of gene flow (Snow 2002). Additional knowledge about grass ecology and biology, population genetics, and potential effects in complex and diverse natural ecosystems is needed.

Uncertainty about gene flow might be balanced by well-designed experiments or management practices (FAO/WHO 2001; Wilkinson 2002). Unfortunately, there is a limit to the ability of small-scale experiments (greenhouse or field trials) to predict consequences of large-scale production and use. Although mathematical models may be useful, modeling may never provide a high degree of confidence about risk for species and ecosystems outside the parameters on which the model is based (Kareiva, Parker, and Pascual 1996; Slavov, Difazio, and Strauss 2002). The development of theoretical approaches to study the potential hazards and frequency of gene flow in perennial grasses is difficult because genes from grasses can be introduced into other populations of grasses by pollen, seeds, or plants. Therefore, questions about the risk of BD grasses and gene flow remain unanswered.

Although rigorous evaluations of BD plants, including grasses, must be conducted by government agencies, there are economic costs associated with regulatory review (NAS–NRC 2000). If the burden of regulation becomes too high, it will impede development of new products, new technologies, and investments in biotechnology. Participation of small companies and academic institutions in the development of BD grasses is inhibited, further concentrating the technology in a small number of companies. The concern is especially acute for public institutions in developing countries, where the benefit of BD forage grasses, such as Napier grass in Kenya, could be significant. Nonetheless, the ecological costs resulting from the release of an invasive BD grass need to be considered and balanced with the potential regulatory costs and benefits of the technology.

Stakeholders and Perceptions of Risk

There are many different stakeholders in the current discussion of BD grasses. These stakeholders include biotechnology companies, grass seed producers, sod producers, golf course managers, athletic-field managers, livestock producers, homeowners, land managers, environmentalists, government officials, the general public, and other groups. With such a large and diverse list of stakeholders, it is easy to understand that differences exist in their identification of potential risks, their perceptions of risks and benefits, and their understanding of the science supporting biotechnology and risk assessment. It is impossible to detail accurately the perspectives of all stakeholder groups. It may be instructive, however, to outline briefly the specific benefits and risks presented by certain stakeholders at the workshop.

Many turf and forage managers see BD grasses as offering new methods to produce healthy grass while decreasing inputs. Benefits from herbicide-resistant BD grasses would include better weed control programs while offering the opportunity to reduce inputs and decrease labor costs. These managers propose stewardship (risk management) and educational programs to manage risks, such as implementation of practices to decrease development of herbicide-resistant weeds.

Certain weed scientists and ecologists consider BD perennial grasses to be "a unique man-made form of biological pollution" aimed at the large commercial and homeowner markets (ICTA 2003). They are concerned about negative environmental impacts including contaminating native and nonnative grass species with transgenes, increasing use and misuse of herbicides, increasing *herbicide resistance* in other weeds, and creating other indirect environmental and economic impacts. They emphasize that the biology of turfgrass species and widespread distribution across the United States could create potentially irreversible problems. As a result, some argue for intense management practices, some argue for strict regulation, and others argue that BD varieties should

not be introduced at all.

Companies that produce grass seed, especially in Western states such as Oregon, have their own perspective on BD grasses. Certain seed companies are concerned about the potential of gene flow into production fields with traditional, non-BD cultivars. They want to know who will be liable for transgene escape, whether seed lots will be tested, and what the acceptable level of contamination will be. There also is concern about the potential effect that gene flow will have on the import and export of grass seed. Certain seed producers think it is impossible to contain genes, whereas others think that implementation of stewardship programs can manage the risks and create a fair situation for all seed producers.

One message from many workshop participants was the importance of stewardship and risk management tools to protect all stakeholders if BD grasses are deregulated. Suggested management practices included the use of *isolation distances* between grass seed fields to prevent gene flow, and the use of herbicides with different *modes of action* to prevent the selection of herbicide-resistant weeds. Management practices should be flexible and may need to change through time, such as when herbicides are introduced or removed from the market. Certain participants supported program monitoring to determine the effectiveness of stewardship programs, improve understanding of the behavior and possible long-term impacts of BD plants, and identify unexpected problems at an early stage. Monitoring criteria and guidelines could be developed collaboratively with ecologists and evolutionary biologists as well as with industry scientists. It is unclear, however, who would conduct the monitoring programs or what criteria would be used.

Conclusions

The workshop provided a valuable opportunity to integrate current knowledge and opinions about the regulatory, scientific, economic, political, and social implications of BD grasses. Even though a few individuals felt strongly that plant biotechnology has outpaced existing government regulations, there was a diverse range of opinion on biotechnology and its application to turf and forage grasses. The workshop demonstrated, however, that a dialogue was possible to help develop a research agenda, systematic approaches to predicting hazards, methods for quantifying risk and benefits, and programs for stewardship. In part, these efforts can be based on the widely accepted concepts of evaluation on a case-by-case basis, the principle of familiarity, and the importance of credible, transparent scientific information for decision making.

Biotechnology-derived grasses are one product from more than 20 years of plant biotechnology research. It is likely that science and technology will continue to alter the methods for developing new grass varieties. New technologies may be able to address specific concerns about BD grasses such as decreasing the risk of gene flow by the use of plant sterility mechanisms; however, these mechanisms are not absolute and would require the incorporation of transgenes using biotechnology. In addition, information from the disciplines of *plant genomics*, *proteomics*, and *metabolomics* is being applied to crop improvement programs. Undoubtedly, government regulations and the science of risk analysis will have to respond to each new step in crop improvement.

3 Background Information on Perennial Grasses

Overview of the Perennial Grass Industry

Perennial grasses are an extremely important part of modern life. This chapter introduces the diverse range of perennial grasses specifically cultivated to benefit humankind. The use and benefits of perennial grasses are subtle and often go completely unnoticed by the general public. This overview will survey the turf and forage grass industries along with details on grass biology and its effect on the deregulation and release of biotechnology-derived (BD) grasses. Information on conventional plant breeding techniques currently in use to develop improved turf and forage grasses also will be discussed.

Perennial grasses are divided into turf and forage, both of which play significant roles in American agriculture (Table 3.1). In fact, several reports have indicated that turf, or what is commonly referred to as the "Turfgrass Industry," is the fastest-growing segment of U.S. agriculture. The turfgrass industry usually is thought to consist of four separate components: (1) golf courses, (2) sports fields, (3) lawns (including commercial lawn care), and (4) sod production. Each component contributes significantly to the overall impact of turf on the U.S. economy. On the other hand, forage, pasture, and range grasses comprise the largest number of acres for any crop in the United States. Perennial grasses are an economically and environmentally significant set of species in our urban, suburban, and agricultural landscapes.

To understand the role of perennial grasses in agriculture and landscapes, the size and value of various industries related to these grasses first must be described. Then it is possible to summarize the range of management regimes required to provide the correct perennial grass for the job at hand. Where and how grass seed and sod are produced for various species also will be examined. This chapter provides current statistics and background about both the turf and forage grass industries, discusses the importance of these grasses, and explains how scientists are able to use available technology to respond to conservation, environmental, and public concerns.

Industry Size and Value in the United States

Because of the wide variety of uses of perennial grasses, it is difficult to estimate accurately the size and value of the industry. There are approximately 17,000 golf courses in the United States, with more than 200 being built each year (NGF 2003). It has been estimated that golf courses themselves represent a $6 to $8 billion dollar industry (Snow 1993). Sports fields include more than 30,000 facilities representing 700,000 individual fields (King 2002). Again, these numbers continue to grow each year. Yet the number of individual detached homes in the United States dwarfs the number of golf courses and sports fields. In 2000, there were an estimated 70 million detached homes (U.S. Census Bureau 2000) with more than 1 million new homes being built each year (U.S. Census Bureau 2002). More than $22 billion dollars is spent annually on lawn care (Packaged Facts 2003). In addition, the number of new homes being built each year has fueled the expansion of U.S. sod-production operations. Sod production represents more than approximately 121,500 hectares (ha) (300,000 acres [a.]) and is a more than $800 million industry (USDA–ERS 2002). When these components are considered collectively, the economic value of turfgrass is estimated at $40 billion. In Maryland, Pennsylvania, Florida, New Jersey, and North Carolina, turfgrass is either the number one or number two commodity in the state. Some believe this estimate is low, but most agree that the industry is expected to show continued growth in the future.

As with the turfgrass industry, forages have a significant economic impact. Pasture, grassland, and rangeland encompass more than approximately 349.8 million ha (864 million a.) in the United States (USDA–NRCS 1997). Forages support the following industries: beef, dairy, sheep, goats, swine, horses, and poultry, as well as various types of wildlife including deer, birds, and wildfowl. Livestock and forage production on grazing lands is the basis for an agricultural industry responsible for $40 billion in agricultural income from 100 million domestic ruminants annually (USDA–NASS 2001). The American

Table 3.1. Summary of perennial grasses in North America

Latin name	Common name	Usage[a]	Type[b]	Humidity[c]	NNA[d]	Wild[e]	Reprod[f]	Tillering[g]
Agropyron cristatum (L.) Gaertn.	Crested wheatgrass, fairway	RT	Cool	Dry	No	Yes	S	I
Agropyron desertorum (Fisch. ex Link) Schult.	Crested wheatgrass, standard	R	Cool	Dry	No	Yes	S	I
Agrostis canina L.	Velvet bentgrass	T	Cool	Humid	No	Yes	S	R
Agrostis capillaris L.	Colonial bentgrass	T	Cool	Humid	No	Yes	S	S/R
Agrostis castellana Boiss. & Reuter	Dryland bentgrass	T	Cool	Both	No	Yes	S	R
Agrostis gigantea Roth	Redtop	F	Cool	Humid	No	Yes	S	R
Agrostis idahoensis Nash	Idaho bentgrass	T	Cool	Both	Yes	Yes	S	I
Agrostis stolonifera var. *palustris* (Huds.) Farw.	Creeping bentgrass	T	Cool	Humid	No	Yes	V/S	R
Alopecurus arundinaceua Poir.	Creeping foxtail	F	Cool	Humid	No	No	S	R
Alopecurus pratensis L.	Meadow foxtail	F	Cool	Humid	No	No	S	I
Andropogon gerardii Vitman	Big bluestem	FR	Warm	Both	Yes	Yes	S	I
Bothriochloa caucasica (Trin.) C.E. Hubbard	Caucasian bluestem	F	Warm	Both	No	No	S	I
Bothriochloa ischaemum (L.) Keng	Yellow bluestem	F	Warm	Both	No	No	S	I
Bouteloua curtipendula (Michx.) Torr.	Sideoats grama	FR	Warm	Both	Yes	Yes	S	R
Bouteloua gracilis (H.B.K.) Lag. ex Steud.	Blue grama	FR	Warm	Both	Yes	Yes	S	I
Bromus inermis Leyss.	Smooth bromegrass	F	Cool	Both	No	Yes	S	R
Bromus wildenowii Kunth	Prairiegrass	F	Cool	Humid	No	Yes	S	I
Buchloe dactyloides (Nutt.) Engelm.	Buffalograss	RT	Warm	Dry	Yes	Yes	V/S	S
Cynodon dactylon (L.) Pers.	Bermudagrass	FT	Warm	Humid	No	No	V/S	S
Dactylis glomerata L.	Orchardgrass	F	Cool	Humid	No	No	S	I
Deschampsia caespitosa (L.) Beauv.	Tufted hairgrass	FT	Cool	Humid	Yes	Yes	S	I
Dichanthium annulatum (Forsk.) Stapf	Old World bluestem	F	Warm	Both	No	No	S	I
Dichanthium aristatum (Poir.) C.E. Hubbard	Old World bluestem	F	Warm	Both	No	No	S	I
Elymus canadensis L.	Canada wildrye	F	Cool	Humid	Yes	Yes	S	I
Elymus dahuricus Turcz. ex Griseb.	Dahurian wildrye	R	Cool	Dry	No	Yes	S	I
Elymus lanceolatus (Scribn. & J.G. Smith) Gould	Thickspike wheatgrass	R	Cool	Dry	Yes	Yes	S	R
Elymus sibiricus L.	Siberian wildrye	R	Cool	Dry	No	Yes	S	I
Elymus trachycaulus (Link) Gound ex Shin.	Slender wheatgrass	R	Cool	Dry	Yes	Yes	S	I
Elymus virginicus L.	Virginia wildrye	F	Cool	Humid	Yes	Yes	S	I
Elytrigia repens (L.) Nevski	Quackgrass	F	Cool	Humid	No	Yes	V/S	R
Eragrostis curvula (Schrad.) Nees	Weeping lovegrass	F	Warm	Both	No	No	S	I
Eragrostis lehmanniana Nees	Lovegrass	R	Warm	Dry	No	No	S	I
Eremochloa ophiuroides (Munro) Hack.	Centipedegrass	T	Warm	Humid	No	No	V/S	S
Festuca arundinacea Schreb.	Tall fescue	FT	Cool	Humid	No	No	S	I
Festuca gigantea (L.) Vill.	Giant fescue	F	Cool	Humid	No	No	S	I
Festuca ovina L.	Sheep's fescue	T	Cool	Humid	No	Yes	S	I
Festuca ovina var. *duriuscula* (L.) Koch	Hard fescue	T	Cool	Humid	No	Yes	S	I
Festuca pratensis Huds.	Meadow fescue	F	Cool	Humid	No	Yes	S	I
Festuca rubra L. ssp. *commutata* Gaud.	Chewings fescue	T	Cool	Humid	No	Yes	S	I
Festuca rubra L. ssp. *rubra* Smith	Creeping red fescue	T	Cool	Humid	No	Yes	S	R

Latin name	Common name	Usage[a]	Type[b]	Humidity[c]	NNA[d]	Wild[e]	Reprod[f]	Tillering[g]
Holcus lanatus L.	Yorkshire fog	F	Cool	Humid	No	No	S	I
Holcus mollis L.	German velvetgrass	F	Cool	Humid	No	No	S	R
Leymus angustus (Trin.) Pilger	Altai wildrye	R	Cool	Dry	No	Yes	S	R
Leymus cinereus (Scribn. & Merr.) A. Love	Basin wildrye	R	Cool	Dry	Yes	Yes	S	I
Lolium hybridum Hausskn.	Hybrid ryegrass	F	Cool	Humid	No	No	S	I
Lolium multiflorum Lam.	Italian ryegrass	F	Cool	Humid	No	No	S	I
Lolium perenne L.	Perennial ryegrass	FT	Cool	Humid	No	No	S	I
Oryzopsis hymenoides (Roem & Schult.) Ricker	Indian ricegrass	R	Cool	Dry	Yes	Yes	S	I
Panicum coloratum L.	Kleingrass	R	Warm	Dry		Yes	S	
Panicum virgatum L.	Switchgrass	FR	Warm	Both	Yes	Yes	S	R
Pascopyrum smithii (Rydb.) A. Love	Western wheatgrass	R	Cool	Dry	Yes	Yes	S	R
Paspalum dilatatum Poir.	Dallisgrass	F	Cool	Humid	No	No	S	I
Paspalum notatum Fluegge	Bahiagrass	FT	Cool	Humid	No	No	V/S	S
Paspalum vaginatum Swartz	Seashore paspalum	T	Cool	Humid	No	No	V/S	S/R
Pennisetum ciliare (L.) Link	Buffelgrass	R	Warm	Both	No	No	S	R
Pennisetum clandestinum Hochst. ex Chiov.	Kikuyugrass	FT	Warm	Humid	No	No	V/S	S/R
Pennisetum flaccidum Griseb.	Flaccidgrass	F	Warm	Both	No	No	V/S	I/R
Pennisetum purpureum Schumach.	Elephantgrass	F	Warm	Humid	No	No	S	I
Phalaris aquatica L.	Phalaris	F	Cool	Humid	No	No	S	R
Phalaris arundinacea L.	Reed canarygrass	F	Cool	Humid	Maybe	No	V/S	R
Phleum bertolonii DC	Turf timothy	T	Cool	Humid	No	No	S	C
Phleum pratense L.	Timothy	F	Cool	Humid	No	No	S	C
Poa annua L.	Annual bluegrass	T	Cool	Humid	No	Yes	V/S	I
Poa arachnifera Torr.	Texas bluegrass	FT	Cool	Humid	Yes	Yes	S	S
Poa pratensis L.	Kentucky bluegrass	FT	Cool	Humid	Maybe	Yes	V/S	R
Poa supina Schard.	Supina bluegrass	T	Cool	Humid	No	Yes	V/S	S
Poa trivialis L.	Rough bluegrass	T	Cool	Humid	No	Yes	V/S	S
Psathrostachys juncea (Fisch.) Nevski	Russian wildrye	R	Cool	Dry	No	Yes	S	I
Pseudoroegneria spicata (Pursch) A. Love	Bluebunch wheatgrass	R	Cool	Dry	Yes	Yes	S	I
Puccinellia distans (L.) Parl.	Slender alkaligrass	FT	Cool	Humid	Yes	Yes	S	I
Schizachyrium scoparium (Michx.) Nash	Little bluestem	FR	Warm	Both	Yes	Yes	S	I
Sorghastrum nutans (L.) Nash	Indiangrass	FR	Warm	Both	Yes	Yes	S	I
Stenotaphrum secundatum (Walter) Kuntze	St. Augustinegrass	T	Warm	Humid	No	No	V/S	S
Stipa viridula Trin.	Green needlegrass	R	Cool	Dry	Yes	Yes	S	I
Thinopyrum intermedium (Host) Barkw. & D.R. Dewey	Intermediate wheatgrass	R	Cool	Dry	Yes	Yes	S	R
Thinopyrum ponticum (Podp.) Barkw. & D.R. Dewey	Tall wheatgrass	R	Cool	Dry	Yes	Yes	S	I
Tripsacum dactyloides (L.) L.	Eastern gamagrass	FR	Warm	Both	Yes	Yes	S	R
Zoysia japonica Steud.	Zoysiagrass	T	Warm	Humid	No	No	V/S	R

[a]Usage: F = forage, R = range, T = turf.

[b]Type: Warm or Cool.

[c]Humidity: Dryland, Humid, Both.

[d]NNA: Native to North America.

[e]Wild: With “close” wild relatives native to North America.

[f]Reprod (Method of reproduction): V = vegetative is possible, S = seed only.

[g]Tillering: I = intravaginal only, R = rhizomes, S = stolons, C = corms.

Table 3.2. Comparison of the size and value of turfgrass and hay with corn and wheat

Crop	Hectares (millions)	Value, $ (billions)
Corn	27.9	19.2
Wheat	19.7	5.6
Hay	25.7	12.6
Turf	20.2	40.0

Forage and Grassland Council (2001) estimates that the forage-livestock industry contributes more than $60 billion in farm sales annually, and the $11 billion hay crop is the third most valuable crop after corn and soybeans (AFGC 2001). Table 3.2 compares the size and value of corn, wheat, hay, and turf crops (Morris 2003).

Turf and Forage Grass Distribution within U.S. Climatic Zones

Climate is a dynamic combination of environmental factors that influences the growth and development of turfgrasses; these factors include light, temperature, precipitation, and wind. Temperature extremes and precipitation patterns are the most significant determinants influencing the range in turfgrass species adaptation (Beard 2002). Cool-season turfgrasses grow best at soil temperatures between 16 and 24°C (60 to 75°F). In contrast, warm-season turfgrasses grow best in soil temperatures between 27 and 35°C (80 to 95°F) (Figure 3.1). Table 3.3 summarizes the distribution of turfgrasses generally used in the various climatic and cultural regimes of the United States. Figure 3.2 depicts the geographical distribution of turfgrass species in relation to the major climatic zones.

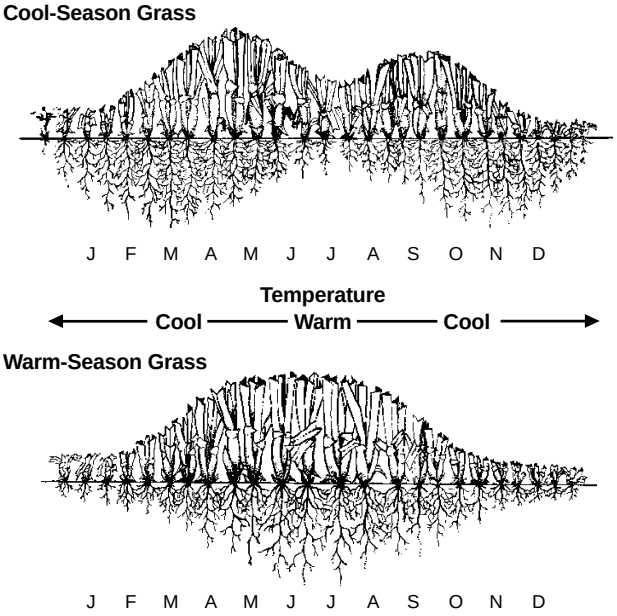

Figure 3.1. Seasonal shoot and root growth for cool-season and warm-season grass species (from Turgeon 1996). Cool-season turfgrasses perform better during spring and fall when temperatures are between 60 and 75°F (16 to 24°C). In contrast, warm-season turfgrasses grow best in summer when temperatures are between 80 and 95°F (27 to 35°C).

Table 3.3. Turfgrass species used in various climatic and cultural regimes within the United States (Adapted from Beard 2002)

		Climate		
Cultural intensity	Extent of use	Cool	Warm	Arid
Low-intensity maintenance	Wide	Kentucky bluegrass Chewings fescue Hard fescue Red fescue Perennial ryegrass	Common bermudagrass	Common bermudagrass
	Limited	Sheep fescue Colonial bentgrass Tall fescue	Carpetgrass Centipedegrass Kikuyugrass Seashore paspalum St. Augustinegrass Zoysiagrass	Blue grama Buffalograss
Moderate-intensity maintenance (irrigation plus close mowing)	Limited	Kentucky bluegrass Perennial ryegrass	Hybrid bermudagrass Common bermudagrass	Hybrid bermudagrass Common bermudagrass

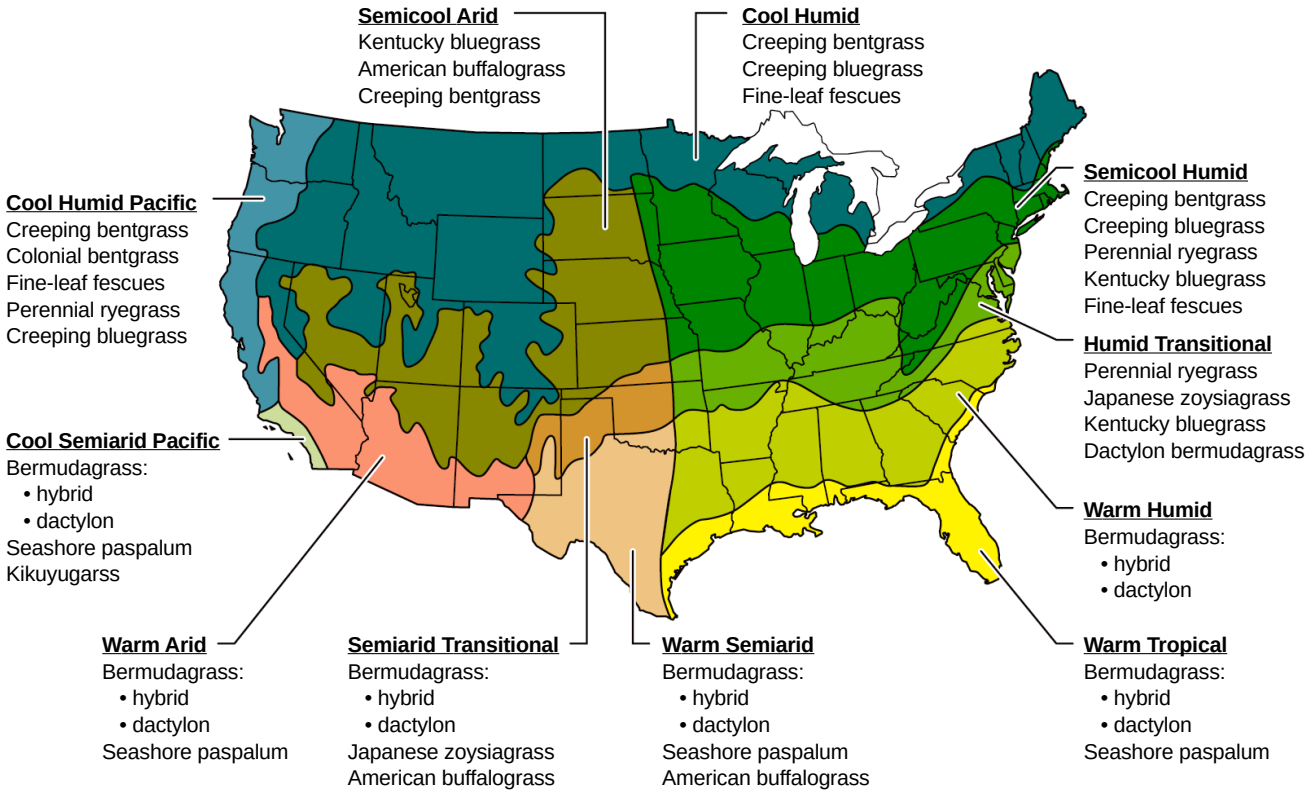

Figure 3.2. Major turf climatic zones and geographic distribution of species in the United States (adapted from Beard 2002).

Turf and Forage Grass Management

The management of turf and forage grasses involves five basic management practices: mowing, harvesting or grazing, fertilization, irrigation, pest control, and cultivation. The extent or frequency of each of these practices is determined by how the turfgrass or forage is used. For example, managing a golf course is considerably different from managing a residential lawn. Likewise, producing hay or silage requires many more management inputs than grazing native range. Tables 3.4 and 3.5 compare the various management practices for two uses of turfgrasses and for three types of forages.

Table 3.4. Comparison of intensity of management practices between a golf course and a home lawn

Practice	Golf course	Home lawn
Fertilization	Biweekly (light amounts)	Once or twice/year
Mowing	Daily (greens)	Weekly
Irrigation	Daily	Occasionally
Pest control	Often	Occasionally
Cultivation	Many times/year	Rarely

Seed Production

Seed of various cool-season grasses (e.g., tall fescue, perennial ryegrass) is produced primarily in the Pacific Northwest. Grass seed was harvested from more than 194,331 ha (480,000 a.) in Oregon and Washington in 2001; production was valued at more than $300 million (OASS 2002; WASS 2002). Seed of certain warm-season grasses, mainly bermudagrass, is produced predominantly in Yuma County, Arizona, and the Imperial Valley of California. Seed

Table 3.5. Comparison of intensity of management practices among hay/silage production, pastureland, and rangeland

Practice	Hay/Silage	Pastureland	Rangeland
Harvests	1–10	1–10	1–2
Fertilization	Frequent	Infrequent to frequent	Infrequent to none
Irrigation	Frequent to none	Infrequent	Infrequent
Pest control	Frequent to occasional	Occasional	Infrequent
Cultivation	Frequent to occasional	Occasional	Infrequent to none

of several native grasses such as big bluestem, switchgrass, and buffalograss, is produced in the Midwest. Forage grass seed is used primarily in the central and southern United States, whereas turfgrass seed is used throughout the United States on golf courses, home lawns, athletic fields, roadsides, and sod farms.

Cool-Season Seed Production

The majority of cool-season grass seed production takes place in the Pacific Northwest, and the seed is shipped worldwide. Seed is harvested only once a year. The seed fields are planted in spring or late summer to early fall, and with the exception of the bentgrasses, which mature in August, most crops are harvested in early July. The seed of fall-planted Kentucky bluegrass is harvested in 11 months, but if planting is in the spring, the first harvest occurs in 16 months. Bentgrass usually is planted in the spring, and it takes about 16 months before the first harvest in August. It is common to spray seed production fields with herbicides in the fall before planting and again in the spring before emergence for spring-planted varieties. Fields are planted as seeded or vegetative rows, and the average field size for each variety is approximately 24.3 ha (60 a.). Many production fields do not receive irrigation; irrigated fields receive at least one fall and two spring irrigations. The number of irrigations depends on the climatic conditions and the species. For example, Kentucky bluegrass can be irrigated up to eight times in semiarid regions (Samudio, S. 2003. Personal communication).

Most fields receive split fertilizer applications in the spring and fall. Weed management strategies usually are initiated in the spring. Fungicides and insecticides are applied as needed. After harvest, fields must be cleaned to ensure good yields in subsequent years; residue management is important. Burning the fields helps to decrease insect and disease problems, and with Kentucky bluegrasses, burning stimulates floral development. In addition to management by burning, straw usually is baled and removed, and the field then is either burned or harrowed followed by a flail chopper. Depending on the species and other factors, seed fields can remain economically viable for up to 7 years (Samudio, S. 2003. Personal communication). For example, perennial ryegrass has the shortest field life expectancy of 3 years. Typically, old seed fields are treated with herbicides, plowed, and fallowed or planted to other crops to control volunteer grass plants.

Warm-Season Seed Production

Bermudagrass seed production takes place primarily in the Southwest. Fields are broadcast planted in late May to June into flat seedbeds that usually are leveled precisely to allow for flood irrigation. Fields usually are preirrigated and broadcast seeded, then watered every 3 to 5 days until the grass seedlings are established (five to seven times). Weed infestation is heaviest in the establishment year. A seed crop is harvested in late June, and a December seed crop is possible; however, most farmers manage the fields for summer hay. Bermudagrass requires 5 acre-feet (1 acre-foot = 325,848 gallons) of water per acre per year. The number of irrigations per year varies but generally includes two heavy irrigations during the winter. Bermudagrass responds well to nitrogen (N), and each seed crop requires approximately 125 lbs. of actual N per acre. Fields intended for summer hay production require more fertilizer. Phosphate is added occasionally as needed. Field start-up is in early March with N fertilizer and water. Weeds usually are chemically controlled with herbicides, and insecticides and fungicides are used occasionally. Fields are baled and cleaned after harvest. A bermudagrass seed production field will remain in production for approximately 5 years.

Even though most zoysiagrass cultivars are produced vegetatively (sod), there is a small amount of seed production primarily in the Southeast. Fields are planted in late spring into weed-free seedbeds and kept moist until established. Approximately 1 month after planting they are fertilized with a balanced fertilizer (N:P:K ratio equal to 3:1:2). During the first year, they are watered and mowed to promote fill-in. Weed control is practiced during winter dormancy, followed by spring preemergent applications and spot spraying for weeds during the growing season. Mole crickets can be a problem on certain fields, and insect hot spots are treated. Fields usually are spring fertilized and a seed crop harvested in early to mid-June; after harvest, fields are maintained by mowing. Zoysiagrass fields can be harvested either as seed or sod. Zoysiagrass has hard seeds, and less than 5% will germinate without seed treatment. Seeds usually are chemically treated to remove waxy layers from the florets; as an alternative, the seed florets can be removed by hulling.

Native grass seed production has several problems, including inherent low yields, harvest difficulties, weed competition and control, insect pests, and after-harvest seed dormancy. Low yields and harvesting difficulties are of primary importance, but

many native grasses, such as buffalograss, have dormant seeds that require exposure to low temperature and chemical treatments before they can be used for turf or forage establishment. Low yield and seed dormancy increase the seed costs of these grasses.

Biology of Perennial Grasses

A discussion of perennial grasses requires knowledge of their basic biology. Grasses are a highly adapted and unique group of plants with plant parts and growth habits not typically found in other plants. The form and function, reproductive characteristics, and growth cycle of perennial grasses each will be reviewed. The diversity among perennial grasses also will be presented, with a discussion on how grasses have adapted to the selection pressures imposed by animals and humans.

Origins and Evolution

The grasses generally are considered to be one of the most recent taxonomic branches of flowering plants and represent an extreme form of specialization that is highly simplified in both vegetative and reproductive form (Stebbins 1972). The first verifiable fossils of grasses occur in mid-Tertiary (more than 30 million years ago) sediments and consist of seeds that are similar in appearance to those of modern grasses such as *Stipa*, *Piptochaetium*, and *Phalaris* (Beetle 1958; MacGinitie 1953). Although fossils that seem to have grass leaves have been found in strata as old as the Cretaceous period (80 million years ago), they cannot be verified as grasses (Stebbins 1972). Although the earliest grasses certainly originated before the Tertiary period, nothing is known of their *morphology* or *taxonomy*.

The evolution of turf and forage grasses took on added significance and value after the last Ice Age. Fossil records suggest that evolution of the extensive North American grasslands largely was responsible for the great evolutionary advances in the horse, including increase in body size, strengthening of teeth, and loss of toes (Thomasson 1979). Likewise, perennial grasses have evolved numerous defense mechanisms to survive and/or thrive under grazing pressure, including *axillary meristems*, *rhizomes*, *stolons*, *trichomes*, *siliceous dentations*, *alkaloids*, *phenolic compounds*, and associations with *endophytic fungi* (Casler et al. 1996). Many grasses have become dependent on herbivores and need to be grazed often to survive (McNaughton 1979). Perennial grasses respond to grazing by increasing their photosynthetic rate, leaf growth rate, and protein concentration, making them more desirable and nutritionally valuable to grazers (McNaughton, Coughenour, and Wallace 1982). The evolution of grasslands partly may have directed the evolution of humans, forcing the upright gait, tool-using hands, and heightened intellect required for survival in such a demanding habitat (Pohl 1987). If true, this represents an interesting irony—humans now are influencing the evolution of grasses and grasslands.

The grass family consists of more than 651 genera, approximately 10,000 species, grouped into six subfamilies and 40 tribes (Clayton and Renvoize 1986). Many of the most important food, fiber, and feed plants are domesticated grasses. *Domestication* is evolution under human influence (Harlan 1975). Cereal grains such as wheat (*Triticum aestivum*) and rice (*Oryza sativa*) have been highly domesticated from their wild forms, a result of thousands of years of genetic mutations and subsequent selection by humans. In a strict sense, domesticated species are phenotypically distinct from their wild forms, such that domesticated forms clearly are more useful to humans (Isaac 1970). Harlan (1992), however, more liberally defines turf and forage grasses as domesticated crops because they generally cannot reproduce themselves, true to form, without the efforts of humans.

With one exception, perennial forage grasses are not domesticated based on the strict definition of Isaac (1970); "wild" collections rarely can be phenotypically distinguished from cultivated forms. The single exception is Italian ryegrass (*Lolium multiflorum* Lam.), which was developed by *unconscious selection* before the twelfth century in the Lombardy and Piedmont plains of Italy (Beddows 1953). Ryegrass (*Lolium* spp.) once existed as a "huge hybrid swarm," with perennial and Italian phenotypes representing opposite extremes of a continuum (Tyler, Chorlton, and Thomas 1987). Hay harvesting, followed by reseeding with shattered seed, resulted in the tall, sparsely tillered, semiannual phenotype that since has been elevated to species status (Breese and Tyler 1986).

Perennial ryegrass (*Lolium perenne* L.) represents the opposite extreme found within the archaic *Lolium* hybrid swarm. Perennial ryegrass seems specifically adapted to survive in association with large herbivores (Beddows 1953; Breese 1983) and is found rarely in ungrazed natural habitats (Davies et al. 1973). Perennial ryegrass seems to have spread throughout the Mediterranean Basin in direct association with the development and spread of livestock

agriculture during human migration (Balfourier, Imbert, and Charmet 2000).

Because of recent efforts by turf breeders, many turfgrasses, in contrast to forage grasses, can be considered to be partly domesticated, even using the strict definition of Isaac (1970). Turf-type perennial ryegrass, with greater shoot density, more diminutive leaves, and shorter flowering stems than forage types, represents a further move toward domestication of this species (Thorogood 2003). Similar phenotypes have been created by mutation, *hybridization*, and selection within several other turfgrasses (Casler and Duncan 2003).

Form and Function

The mature-plant height of grasses ranges from 2 centimeters (cm) to 30 meters (m). They can be either *herbaceous* or *woody* (Hitchcock and Chase 1950). All grasses have three major vegetative organs: root, stem, and leaf. Aboveground tissues are organized into shoots, sometimes called *tillers*. A tiller consists of a hollow whorl of leaves, protecting the *apical meristem* (growing point) from which each leaf originates. In perennial grasses the apical meristem arises near the soil level and consists of a series of compressed nodes. This region of the plant, the interface between roots and shoots, is commonly referred to as the crown of the plant. The bulk of the crown is just below or at the soil surface (Figure 3.3).

Each grass leaf consists of two structures: a sheath and a blade (or *lamina*). The sheath and blade are separated by a collar that may have additional structures, such as a *ligule* (a hairy or membranous appendage at the junction of the sheath and blade) or *auricles* (a pair of appendages on either side of the collar). Grass leaves grow from an *intercalary meristem*, a region of cell division at or near the collar. The sheath remains in a round or elliptical shape, forming a hollow tube into which the elongating stem grows. The blade expands, forming new cells near its base, opening up into the typical grass-leaf shape: flat and narrow, with parallel veins. The principal functions of grass leaves are photosynthesis and transpiration (e.g., the exchange of atmospheric gases and water).

Multiplication and differentiation of cells from the apical meristem lead to formation of the stem (or *culm*). A grass stem is a tube that typically is hollow, but may be pith-filled (e.g., maize, *Zea mays* L.), and typically is round, but may be elliptical or flattened (e.g., annual bluegrass, *Poa annua* L.). The tube is interrupted by thickened sections, called *nodes*. A node is the point of attachment for the base of each leaf sheath (Figure 3.3). Each node gives rise to one leaf, and the leaves always are borne in two ranks on the stem (i.e., alternately on opposite sides of the stem). The nodes of many perennial grasses also contain an *axillary bud* that can grow to produce a new shoot and, when placed in contact with soil, produce roots and a "daughter" plant (Casler and Hovin 1980). The stem region between nodes is called the *internode*. Grass stems are divided into *phytomers;* each phytomer contains a node, internode, and leaf. Grass stems generally elevate the leaves above the ground, providing greater light interception for the entire shoot.

Grass stems also elevate the *inflorescence* (the grass flowering structure) above the soil, providing seeds with both a measure of protection from *herbivory* (being eaten) and disease, as well as allowing for a greater opportunity for seed dissemination. A single grass flower, or floret, typically consists of both male and female parts (perfect) or may be exclusively male or female (imperfect). Most perennial grasses have perfect flowers. The floret is enclosed between two modified leaves, the *lemma* and *palea* (Figure 3.4). Each floret produces one *caryopsis*, commonly referred to as a seed. The lemma and palea are fused to the caryopsis in certain species. Florets are borne in spikelets, typically from one to seven florets per spikelet, some of which may be infertile. Spikelets are grouped in clusters on the inflorescence, in various structural arrangements (*raceme*, *panicle*,

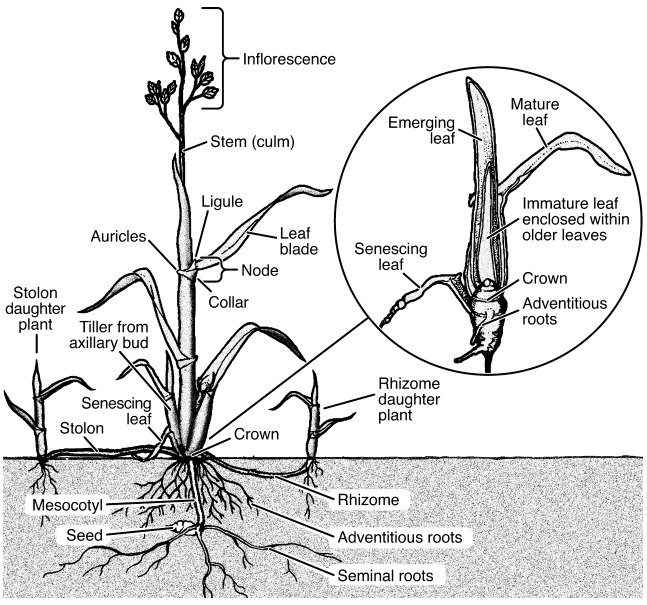

Figure 3.3. Parts of the grass plant and cross-section of the crown with the organization of leaves (adapted from Turgeon 1996).

spike, or compound forms; see Figure 3.5).

Grass root systems are fibrous, highly branched, and consist almost entirely of secondary and *adventitious roots* (secondary roots originating from the crown or nodes). Roots may originate from lower nodes of the stem or from stem nodes (with axillary buds) that come in contact with soil. Roots function as organs for nutrient and water uptake, storage of carbohydrate reserves, and an anchor for the grass plant.

Reproduction

Perennial grasses potentially have three primary reproductive mechanisms: sexual reproduction, cross-pollination, and asexual reproduction. Sexual reproduction occurs by formation of seed after pollination of an ovule with a pollen grain. Cross-pollination is much more common in perennial grasses than self-pollination. Asexual reproduction occurs either via seed or vegetative organs such as stolons and rhizomes (Figures 3.3 and 3.6). Asexual reproduction via seed generally is termed *apomixis* and will be discussed later (see "Reproductive Characteristics").

Vegetative asexual reproduction occurs via one of several specialized organs (Table 3.6). Tillers form when axillary buds (Figures 3.3 and 3.6) on the lower nodes of a grass stem break dormancy and elongate to produce a new shoot. These tillers eventually produce adventitious roots and result in a slow horizontal spread of the plant. Most perennial grasses that reproduce by this mechanism are termed "bunch grasses," because they do not form solid or continuous areas of grass (*swards* or turfs). Perennial ryegrass and tall fescue are examples of two widely used bunch grasses that are used as turf and forage.

Stolons and rhizomes are modified stems that have a similar or identical structure to stems (nodes, internodes, leaves, and axillary buds), but differ significantly in their horizontal growth habit. Leaves on rhizomes or stolons typically are modified in structure, diminutive, or vestigial. Because rhizomes and stolons are highly effective mechanisms for lateral spread over potentially great distances, rhizomatous or stoloniferous grasses such as bermudagrass or Kentucky bluegrass are termed "sod formers."

Stolons originate from axillary buds on nodes at the base of the plant and grow horizontally above the soil surface. Reproduction occurs when adventitious roots form at a node and the axillary bud at that node grows to produce a new shoot, which can further reproduce by tillering and stolon production.

Rhizomes are similar in structure to stolons, except they originate from buds on crown tissue below the surface of the soil and they remain belowground, eventually turning upward and breaking the soil surface to form a new tiller. Why or where a rhizome turns upward toward the soil surface is unknown (Nelson 1996). Roots can form at any node of a rhizome, and rhizomes can have multiple branches, leading to multiple aboveground shoots. Highly ag-

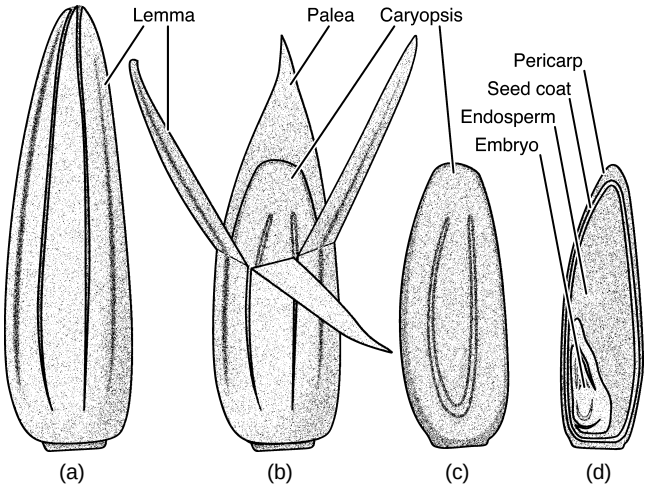

Figure 3.4. Parts of the grass floret: (a) abaxial side showing the lemma; (b) lemma partly removed exposing the palea and caryopsis; (c) caryopsis; and (d) the true seed consisting of a seed coat, endosperm, and the embryo.

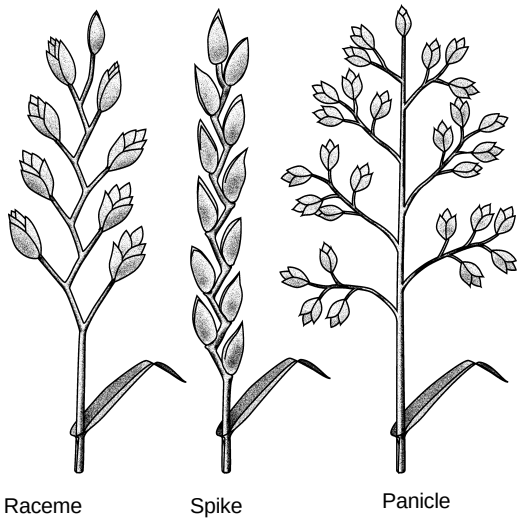

Figure 3.5. Three primary types of grass inflorescences. In the raceme and spike inflorescence types, the main flowering axis is called a rachis, whereas in the panicle the term rachis is applied to the lateral branches (Adapted from Turgeon 1996).

Table 3.6. Common perennial grasses that produce vegetative propagules

Species	Propagule type
Agrostis spp. (Bentgrasses)	Rhizomes or stolons
Bromus ineris (Smooth bromegrass)	Rhizomes
Buchloe dactyloides (Buffalograss)	Stolons
Cynodon dactylon (Bermudagrass)	Rhizomes and stolons
Eremochloa ophiuroides (Centipedegrass)	Stolons
Festuca rubra (Creeping red fescue)	Rhizomes
Paspalum notatum (Bahiagrass)	Rhizomes
Poa pratensis (Kentucky bluegrass)	Rhizomes
Poa trivialis (Rough bluegrass)	Stolons
Stenotaphrum secundatam (St. Augustinegrass)	Stolons
Zoysia sp.	Rhizomes and stolons

gressive species, such as quackgrass, *Elytrigia repens* (L.) Nevski, which is found in cooler climates, have a rhizome whorl that forms a needlelike structure sufficiently sharp to pierce large, fleshy roots that otherwise might impede its progress.

A few perennial grasses such as timothy, *Phleum pratense* L., reproduce by *corms*, fleshy thickenings that form the base of each stem 1 to 2 cm below the soil surface. Corms are a principal storage organ for carbohydrate reserves and reproduce by buds at the base of the corm.

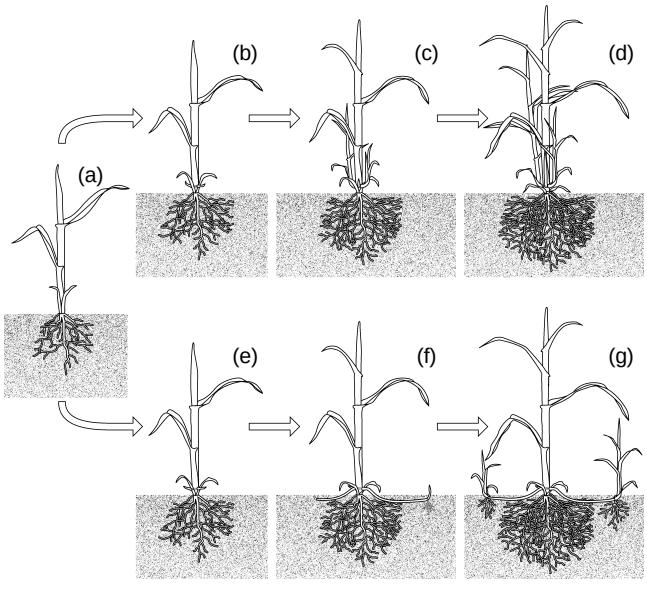

Figure 3.6. Examples of tillering (b–d) and rhizome growth (e–g) branching from a parent shoot (Adapted from Turgeon 1996).

Growth Cycles

Perennial grass plants have three phases in their life cycle: seedling (juvenile), adult-vegetative, and adult-reproductive. Seedling growth and development initially are dependent on carbohydrates and proteins stored in the *endosperm* of the seed. Once sufficient photosynthetic tissue has developed, usually by the two-leaf stage, the seedling becomes *autotrophic* and is able to grow and survive on its own. Autotrophic grass seedlings must pass through a juvenile phase of their life cycle before becoming adult plants. Juvenile plants are unable to respond to environmental stimuli that normally cause physiological changes to the plant, such as *floral induction* and hardening of plants to tolerate stress. The juvenile phase may last from 3 to 6 weeks, depending on species. Adult plants may remain vegetative throughout their life cycle, if they are completely sterile, or may go through an annual flowering cycle that is environmentally and hormonally regulated (Calder 1963).

Most perennial grasses are sensitive to the length of daylight (*photoperiod*)—they respond to seasonal changes in the photoperiod and to changes in photoperiod outside of their original adaptation zone. Most cool-season grasses are long-day plants, whereas most warm-season grasses are short-day plants. Long-day plants flower under relatively long day-lengths, whereas short-day plants flower under relatively short day-lengths. Floral induction of cool-season grasses (*vernalization*) occurs in autumn as days become shorter and temperatures lower. During induction, physiological changes that promote flowering occur in response to colder temperatures. Floral initiation and development occur in spring as days become longer and temperatures rise.

Most perennial grasses have a true dormant phase during winter, but there are certain exceptions. Perennial ryegrass is one of the few cool-season grasses that has no true winter dormancy and as a result has poor tolerance to severe cold temperatures, which decreases its range of adaptation (Breese 1983). Certain warm-season grasses, adapted to tropical climates, will exhibit growth throughout the year. Certain cool-season grasses also have a summer dormancy phase that is dependent on exposure to high temperatures for a sustained period of time. Italian ryegrass is an extreme example of this phenomenon. It is used as a winter annual in the southern United States where high summer temperatures induce dormancy and, eventually, mortality. Although plant breeders have selected plants with this "annu-

al" growth habit, recent survival of occasional plants suggests that decades of natural selection may have selected plants capable of surviving high temperatures.

Perennial grasses synthesize, store, and use carbohydrates in a cyclic process related to both their natural growth cycle and the management system imposed. Carbon fixed during photosynthesis is converted into simple sugars and, in certain species, longer-chain sugars called *fructan*. These soluble carbohydrates are transported and stored in roots and/or stem bases for later use by the plant. Soluble carbohydrates are essential for plants to recover from dormant or semidormant phases of their life cycles, particularly a long overwintering phase. After such a dormant phase, or after grazing or clipping, carbohydrate reserves are essential for new leaf growth and elongation before photosynthesis is sufficient to sustain the metabolic needs of the plant. When leaf area and net photosynthesis reach a critical level, the plant can begin storing soluble carbohydrates for the next regrowth or recovery cycle. Grasses without true winter dormancy, such as perennial ryegrass, continue to respire all winter, using up carbohydrate reserves essential for spring recovery.

Tillering, including rhizome and stolon production, in perennial grasses is an interactive process highly influenced by light, N fertility, and growth stage (Nelson 1996; Nelson and Volenec 1995; Volenec and Nelson 1995). Tillering is stimulated by defoliation, whether by hay harvest, grazing, or mowing. This is thought to be a response to increased light quality at the lowest levels of the grass canopy where new tillers begin their development (Casal, Sanchez, and Deregibus 1987; Volenec and Nelson 1995). Perennial grasses have an equilibrium tiller density in which tiller mortality is balanced by new tillers (Langer 1963; Zarrough, Nelson, and Sleper 1984). Adequate N fertility is required to ensure that the rate of tiller initiation balances the effect of tiller mortality (Nelson and Zarrough 1981). Tillering is under genetic control (Zarrough, Nelson, and Sleper 1984), and plant breeders have increased tiller densities dramatically by conventional methods (inbreeding) in many of the small-stature turfgrasses (Casler and Duncan 2003).

Diversity

Perennial grasses belong to one of two groups: warm-season or cool-season species. Adaptation zones for warm- and cool-season grasses are defined largely by temperature, which is largely determined by latitude and altitude. Temperature is a major factor in defining climatic and hardiness zones, which in turn can be used to describe the adaptive regions for perennial grasses. Warm-season grasses can be adapted to both warm- and cool-season climates. Certain warm-season grasses have excellent cold tolerance, with adaptation as far north as U.S. Department of Agriculture (USDA) Hardiness Zone 3 in the United States (Cathey 1990). Warm-season grasses of the North American tallgrass prairie tend to have the highest levels of cold tolerance among the warm-season grasses. Likewise, certain cool-season grasses can be adapted to warm-season climates, but they eventually succumb to summer heat, resulting in severe dormancy, stand losses, and mortality. Of the cool-season grasses, those from the Mediterranean region, such as tall fescue (Sleper and West 1996), tend to have the greatest heat tolerance.

Within temperature or hardiness zones, there are additional sources of diversity among the perennial grasses. Precipitation, or moisture availability, is the second most important factor defining adaptation of perennial grasses. Perennial grasses adapted to dryland regions have drought tolerance or dehydration-avoidance mechanisms not present in perennial grasses adapted to humid regions. These mechanisms are the result of evolutionary modification of anatomical, morphological, and physiological traits. Grasses with drought tolerance or dehydration-avoidance mechanisms typically have multiple factors that contribute to their adaptation to dry land conditions (Austin 1989; Johnson and Asay 1993).

Adaptation and diversity among perennial grasses also are regulated by several other *abiotic factors*, including day-length (photoperiod), soil pH (acidity), mineral and/or heavy metal content of soils, and air pollutants (Casler et al. 1996). Numerous *biotic factors*, including disease organisms (bacteria, fungi, viruses, and nematodes) and herbivores (insects, birds, and mammals), also contribute to perennial grass diversity. Each of these factors has contributed, in part, to *speciation*, the process of creating and fixing new variability into distinct taxonomic forms that causes them to be recognized as distinct species. In addition, these factors continue to act on a finer scale within plant populations of a single species, resulting in populations or individuals with local adaptation to particular environmental niches or habitats (Casler et al. 1996).

Breeding and Genetics of Perennial Grasses

Perennial turf and forage grasses form a group of diverse plant species that evolved as a component of natural grasslands in different parts of the world. Much genetic variation exists among and within these plant species because of their evolution under different environmental conditions and the selective forces imposed by those variant conditions. Differences in climate, soil, and defoliation pressures from herbivores are the major environmental factors that have imposed interactive selective pressures leading to the vast genetic variation found in perennial grasses. Varieties of these grasses with desired characteristics have been extracted from natural *germplasm pools* for human use as cultured forage and turf. These varieties have been obtained by the discovery of naturally occurring forms and by plant breeding. Breeding improved turf and forage grass varieties has increased food, feed, and fiber, aesthetic enrichment, and protection of the environment (Sleper, Asay, and Pedersen 1989).

The reproductive differences within and among perennial grasses influence the type of breeding procedure used for improvement and the genetic constitution of varieties developed through breeding. The next section reviews the prevalent reproductive characteristics found in perennial grasses, the methods by which varieties are bred, and the types of varieties in commerce.

Reproductive Characteristics

Perennial turf and forage grasses reproduce by seeds that develop through normal sexual processes or by seeds that develop by apomixis. Perennial grass plants also can be propagated clonally by planting bud-bearing tillers, rhizomes, or stolons. Many of the perennial grasses have more than two basic sets of chromosomes, a condition known as *polyploidy*. Inheritance of traits in polyploid plants is more complicated than in *diploid* (two sets of chromosomes) plants because they have more genes that interact to condition individual traits and because there is greater potential for certain genes to mask the effects of other genes (Bingham et al. 1994; Vogel and Pedersen 1993).

Sexual Reproduction

Most perennial turf and forage grasses reproduce sexually and are cross-pollinated, with the pollen dispersed by wind (Burson 1980; Hanson and Carnahan 1956; Hovin 1980; Vogel, Gorz, and Haskins 1989). Very low levels of inbreeding in these naturally outcrossing species is assured by genetic incompatibility between pollen and pistil of the same plant (Brewbaker 1957; Newbigin, Anderson, and Clarke 1993). Self-incompatibility ensures that self-pollination seldom succeeds in fertilizing the female egg. Outcrossing also is facilitated in certain species because the stamens and pistil mature at different times (Lersten 1980). A limited amount of inbreeding is possible in most of the grasses, but it causes genetic defects including loss of vigor and reproductive capability (Vogel and Pedersen 1993). Consequently, it generally is not possible to inbreed intentionally to the extent necessary to develop *homozygous* (carrying the same *alleles* or versions of a gene for a trait) inbred lines as in corn, *Zea mays*. In these outcrossing species, plant populations are heterogeneous, and individual plants are highly *heterozygous* (carrying different alleles for a trait).

Apomictic Reproduction

Apomixis is defined narrowly as asexual reproduction through the seed, referred to as agamospermy or gametophytic apomixis (Bashaw and Funk 1987). Broader definitions of apomixis include reproduction by vegetative parts. Apomixis is a method of reproduction in which *meiosis* and resulting genetic recombination are circumvented in the female. The embryos that are formed from asexually derived eggs have the same genetic constitution as the female parent. Apomixis is widespread in the plant kingdom and is found in many polyploid turf and forage grasses (Bashaw 1975, 1980; Bashaw and Funk 1987; Hanna and Bashaw 1987; Harlan and de Wet 1963). Various types of apomixis have been described that differ in the way the sexual process is bypassed (Bashaw 1980; Gustafsson 1946).

The frequency of apomixis may vary. Thus *obligate apomixis* is a condition in which plants reproduce solely by apomixis, as opposed to *facultative apomixis* in which both sexual and apomictic reproduction occurs within individual plants or among plants within a population (Bashaw 1980). Obligate apomixis produces progeny that are all exact genetic copies of the maternal parent, but plants that reproduce partly by sexual means will produce progeny of variable genotype and phenotype. Individual plants within the facultative group may vary from fully sexual to fully apomictic, with those between reproducing both sexually and by apomixis at

frequencies that can fluctuate with environmental conditions (Bashaw and Funk 1987; de Wet and Stalker 1974; Harlan and Celarier 1961).

The evolutionary advantages of apomixis include the restoration of fertility in hybrid plants and the maintenance of hybrid vigor derived from favorable gene combinations in highly heterozygous plants (de Wet and Stalker 1974). All plant groups that reproduce by apomixis, however, retain some capability for sexual reproduction to ensure evolutionary success (Bashaw and Funk 1987; Harlan and Celarier 1961). Even in plant groups reproducing by obligate apomixis there usually exist in nature numerous variable biotypes that resulted from sexual reproduction at some time. When even one highly heterozygous plant in an obligate apomictic biotype reverts to sexual reproduction, a large amount of genetic variation is made available for natural selection and allows for plant improvement through artificial selection (Bashaw and Funk 1987). Wide crosses between apomictic male plants and sexual plants of distant relatives also produce genetic variation subject to natural selection.

Apomixis limits the ability of the breeder to hybridize plants and to select recombinants among their progeny.

Vegetative Reproduction

Perennial grass plants can be propagated by vegetative parts such as tillers, rhizomes, and stolons. The ability to propagate individual plants clonally is useful in breeding and genetic research because plants can be replicated and grown through time and/or space for purposes of intercrossing and performance testing (Aastveit and Aastveit 1990; Vogel and Pedersen 1993). Clonal propagation allows sexually sterile plants to be maintained. Individual plants of certain species, mainly the sod-forming species such as bermudagrass, *Cynodon dactylon*, can be clonally propagated economically on a commercial scale. In other species, such as bunchgrasses, although individual plants also can be cloned, commercial propagation usually is not economically feasible.

Conventional Breeding Methods

The type of breeding procedure used for cross-pollinated grasses is determined chiefly by the reproductive mode of the breeding population. The complexity of inheritance for the selection trait(s) also is important. Simply inherited traits that are controlled by one or two genes producing discrete phenotypic classes can be fixed easily in a breeding population by selecting and interbreeding desired phenotypes for one to a few generations. Many of the traits targeted for breeding improvement in turf and forage grasses have complex inheritance because they are controlled by many genes, each with small but important contribution to the total phenotypic response. Such traits include biomass yield, seed yield, forage quality, turf quality, and response to many biotic and abiotic stresses. These are called quantitative genetic traits because plant phenotypic values usually are continuous over a range, rather than discrete.

Crossing Methods

Methods for crossing grass plants range from artificial hybridization by *hand-emasculation* (removal of anthers or the male plant part that produces pollen) and hand-pollination to mutual pollination procedures relying on the natural outcrossing and self-sterility to minimize self-pollination (Burson 1980; Fehr 1987; Hovin 1980). Artificial crossing of grasses with perfect flowers by hand-emasculation of anthers and hand-pollination is tedious and time consuming because of the small size of the flowers (Richardson 1958), although it is practical for certain breeding applications. Procedures have been developed, such as placing plants in fog chambers at the time of flowering, to facilitate emasculation (Burton 1948). The crossing of two parent plants, or the random intercrossing of several plants, often is achieved by growing multiple clones of the plants in the field or greenhouse in isolation from other plants with which they might cross.

Recurrent Selection

Recurrent selection is the breeding procedure of choice to improve quantitative traits in populations of sexually outcrossing turf and forage grasses (Allard 1960; Casler 1999; Fehr 1987; Hallauer 1991; Hanson and Carnahan 1956; Sleper 1987; Vogel and Pedersen 1993; Vogel, Gorz, and Haskins 1989). As the name implies, recurrent selection is a procedure by which plants in a breeding population are selected for trait enhancement, and the process is repeated over successive generations. Recurrent selection for a quantitative trait changes the mean of the population for the selection trait while maintaining enough genetic variation in the population to avoid inbreeding depression (Figure 3.7). Mean population performance is increased as a consequence of increased frequencies of the gene alleles (versions of

genes) that confer improvement of the trait in the population. Effective recurrent selection depends on additive genetic variation, derived from the type of gene action in which replacement of one gene allele by another results in a desirable change in the trait phenotype. Positive response to selection in crop plants has been achieved over many generations for different quantitatively inherited traits (Casler 1999; Hallauer 1991; Hallauer and Miranda 1991).

Several recurrent selection schemes have been developed that differ mainly in how plants are evaluated and selected and how generations are advanced (Fehr 1987). Choice of the recurrent selection scheme best suited for a particular breeding program depends on several factors, notably the magnitude of *heritable* (additive) *variation* in the population for the selected trait and the amount of time required to complete a selection cycle. Plants within a population are selected on the basis of their phenotype (phenotypic recurrent selection), on the basis of their breeding value determined by progeny testing (genotypic recurrent selection), or on both. Generations are advanced either by using seed from maternal plant selections after uncontrolled pollination among all plants in the population or by seed resulting from random *intercrossing* of only the selected plants. Controlling just the maternal plants is only half as effective as controlling both male and female plants (Hallauer and Miranda 1991). Intercrossing selected plants of the naturally cross-pollinated grasses usually is accomplished by growing multiple clones of each selected plant in a field nursery isolated from other plants of the same species.

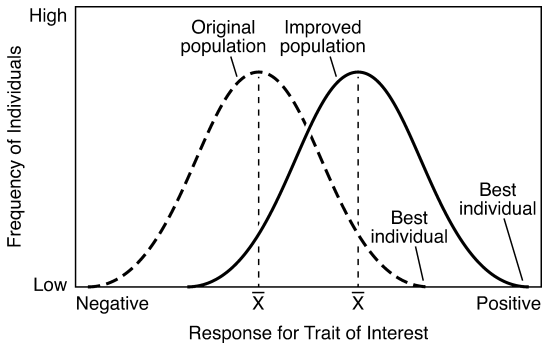

Figure 3.7. Effective recurrent selection results in higher mean performance for the selection trait through successive selection cycles as depicted in this idealized example showing progress from one selection cycle. In addition to higher mean performance, each selection cycle produces certain plants superior to those in previous breeding generations but maintains genetic variation in the breeding population (adapted from Fehr 1987).

Methods for Apomictic Species

Breeding apomictic grasses is possible only if some degree of sexual reproduction occurs in the breeding population, or if related ecotypes (local varieties adapted to a particular environment) or species that reproduce sexually are available and can be hybridized with the apomictic grasses (Bashaw 1975, 1980; Bashaw and Funk 1987; Huff 2003). Breeding apomictic grasses is more complicated than breeding sexually reproducing types (Bashaw 1975, 1980; Bashaw and Funk 1987; Huff 2003). To breed grasses that reproduce by obligate apomixis, related forms with sexual reproduction and hybridization potential are required. Fortunately, such sexual relatives usually exist, although considerable effort may be required to identify them (Hanna and Bashaw 1987). By definition, grasses reproducing by facultative apomixis have some sexual reproduction.

Regardless of the type of apomixis, breeding methodology has been limited largely to hybridization of plants and selection of individual superior plants from the hybrid populations as opposed to population improvement. Both intra-and interspecific hybridizations have been used in breeding apomictic grasses (Bashaw and Funk 1987; Hanna and Bashaw 1987; Huff 2003; Read and Anderson 2003). Manipulation of the growth environment or *ploidy level* of plants can facilitate hybridization in breeding apomictic grasses (Burton and Forbes 1960; Funk and Han 1967; Huff 2003). Because the pollen in apomictic grasses is formed through the normal sexual process, hybridization between obligate apomictic and sexual plants is possible using the former as the male. The highly heterozygous plants produce variable F_1 progeny (first filial generation, or the progeny of an experimental cross) that segregate for many traits, usually including method of reproduction (Bashaw and Funk 1987; Read and Anderson 2003; Read and Bashaw 1969). F_1 hybrid plants normally are segregated on the basis of forage or turf performance traits plus a high degree of apomictic reproduction. Progeny populations also are a source of new plants to use as parents in new crosses (Read and Anderson 2003). Progeny plants that are partly or completely sexual and have desired performance traits often are selected for use as parents. Highly apomictic plants with the desired traits can be used as male parents.

Methods for Vegetatively Propagated Species

The principal breeding method used for vegetatively propagated turf and forage grasses is hybridiza-

tion and selection of elite F_1 progeny plants (Burton 1947, 1959, 1991; Sleper 1987). This simple and direct method of breeding is based on the ability to mass clone the individual hybrid plants. Many elite F_1 hybrid plants will exhibit *heterosis*, or hybrid vigor, because of specific interactions of alleles at the same and different gene loci. These gene interactions may change with sexual reproduction, but clonal propagation captures the genotype in the same manner as apomixis. The breeding success of clonally propagated grasses depends on identifying plants that will produce elite progeny and having an efficient screening procedure to identify the outstanding hybrids. The chances of success also increase in proportion to the number of hybrids produced and evaluated.

Hybrids can be made between closely or distantly related parents, and the progeny plants may be partly or completely sterile. Bermudagrass, *Cynodon* sp., provides a good example of the use of both intra- and interspecific hybridization in clonal variety improvement. Intraspecific hybridization between *C. dactylon* plants was used to produce the landmark "Coastal" and "Midland" pasture varieties planted on several million acres across the southern United States (Burton 1954; Harlan, Burton, and Elder 1954), and *Tiflawn,* a turf bermudagrass (Burton 1991). Hybridization between *C. dactylon* and stargrass (*C. aethiopicus* or *C. nlemfuensis*) was used to enhance growth and forage quality characteristics in hybrid plant varieties such as "Coastcross-1" (Burton 1972; Burton and Monson 1972) and "Tifton 85" (Burton, Gates, and Hill 1993). Hybridization between *C. dactylon* and *C. transvaalensis* resulted in the industry standard triploid (27 chromosomes) sterile "Tifway" and "Tifgreen" turf bermudagrass varieties (Burton 1991), plus a number of other turf bermudagrasses (Taliaferro 2003). Interspecific hybridization in *Cynodon* combined the desirable traits from the respective species into F_1 hybrids.

In addition to hybridization, mutation breeding has been used successfully in the improvement of turf bermudagrass. Powell, Burton, and Young (1974), Hanna (1999), and Hanna, Carrow, and Powell (1997) used Cobalt-60 gamma radiation to produce mutant plants from which they selected superior genotypes.

Types of Varieties

"Variety" and "cultivar" are synonyms that describe plant populations that are distinct, uniform within described limits, and stable during reproduction. The types, or genetic constitutions, of varieties used to propagate perennial turf and forage grasses depend on the kinds of reproductive methods that exist within a plant group discussed earlier. Most, perhaps all, of the current perennial turf and forage grass varieties fit into one of four major varietal categories described by Fehr (1987): (1) open-pollinated, (2) synthetic, (3) line, and (4) clonal. Not included in this list are hybrid varieties. Although many of the grass varieties are F_1 hybrids, they are propagated clonally and fall outside the narrow definition of a hybrid variety as one produced by crossing two inbred parents that produce uniform progeny and exhibit hybrid vigor. Hybrid varieties of perennial grasses have not been developed because of inability to produce inbred lines and the lack of methods such as cytoplasmic male sterility to control pollination. A comprehensive list of turf and forage grass varieties developed in the United States through 1994 is given by Alderson and Sharp (1995).

Open-Pollinated Varieties

Open-pollinated varieties of sexual, outcrossing grasses are characterized by being distinct in one or more traits from other varieties of like kind, but otherwise are genetically diverse. Typically, they are developed by selecting within a genetically diverse random mating population, or among genetically diverse germplasm accessions, for uniformity for one or more traits. Selected germplasm accessions must be combined into a random mating population to qualify as a variety. Selection within open-pollinated varieties usually has been conducted to the minimum extent necessary to achieve a desired performance standard for yield, quality, pest resistance, or adaptation. The extent of genetic variation retained within such varieties is sufficient to prevent appreciable inbreeding depression as generations are advanced. *Mass selection*, a form of selection in which many plants in a population are chosen based on trait phenotype, has been the method of choice to develop open-pollinated varieties. Mass-selected plants usually are chosen as parents to produce the next generation because they express a desired trait. The process also includes the removal (rouging) of plants that express undesirable phenotypes. Vogel and Pedersen (1993) described the general procedures used in developing open-pollinated varieties of cross-pollinated perennial grasses using a system they termed "ecotype selection." The products can be released as commercial varieties or as populations for further breeding.

Most sexually reproducing, seed-propagated, grass varieties developed are open-pollinated. Public agen-

cies such as land-grant institutions, the Agricultural Research Service, and the Natural Resources Conservation Service of the USDA were responsible for developing the bulk of these varieties. These agencies continue to develop open-pollinated grass varieties, but to a lesser extent than in the past.

Synthetic Varieties

Synthetic varieties are developed by intercrossing a specific set of selected clonal plants or seed-propagated lines (Allard 1960; Fehr 1987). The number of parent lines varies from as few as 2 to as many as 15. The distinction between synthetic and open-pollinated varieties is not always sharp. The defining difference is the way the parent lines are chosen (Allard 1960). The parent lines used to produce synthetic varieties are chosen on the basis of their combining ability, which is determined by crossing the lines in all combinations and measuring the progeny performance of all the single crosses. Progeny testing as a measure of the breeding value of a parent is not conducted in the mass selection procedure.

Because of the potential for inbreeding or other appreciable change in the genetic composition of synthetic varieties that might diminish their stability, the number of generations of sexual increase usually is limited to three or four. First-generation (Syn-1) seed is produced by growing the parent lines in isolation using a planting scheme designed to maximize their random intercrossing. For the perennial grasses, clones of parent plants normally are used. In pedigreed seed programs, the Syn-1 generation seed usually serves as Breeder class seed. Seed multiplication is accomplished by producing Foundation (Syn-2 generation), Registered (Syn-3 generation), and Certified (Syn-4) generation pedigreed classes. The Registered class may not be included, depending on the volume of seed needed and the seed-production capability of the variety.

The ability to vegetatively propagate individual plants of certain perennial grasses on a field scale makes first-generation synthetic varieties possible. In such instances, Syn-1 generation seed is produced from fields established by planting in equal proportion clones of the parent lines in mixture. The number of years of seed harvest permitted from such fields may be restricted because of the potential for shifts in the clonal populations.

Line Varieties

Fehr (1987) used Kempthorne's (1957) definition of a line variety as a group of plants of self- or cross-pollinated species that have a theoretical coefficient of parentage of 0.87 or higher, meaning that they are closely related. Line varieties in the context of the cross-pollinated perennial grasses are restricted mainly to those that reproduce by facultative apomixis. Such varieties have been referred to in the literature as "apomictic" varieties rather than "line" varieties (Bashaw and Funk 1987). Fehr (1987) described single line apomictic varieties and noted that classification is complicated by variation among varieties in the percentage of seed produced asexually. He noted that single line varieties with at least 95% apomictic reproduction are considered pure lines. Varieties that reproduce by apomixis as little as 80% of the time were described as meeting the criteria of line varieties, although plants may vary in morphological characteristics because of sexual reproduction.

Clonal Varieties

Clonal varieties represent single plants, or infrequently very similar plants, propagated by vegetative means (Fehr 1987). The term most frequently is used to denote the propagation of a variety by vegetative organs such as tillers, rhizomes, and stolons. Fehr (1987) includes varieties that reproduce by seed produced by obligate apomixis.

Biotechnology Techniques Available for Perennial Grass Breeding

In perennial grasses, *biotechnology,* sometimes referred to as "genetic engineering," involves placing a gene or genes, be it from an animal, a bacterium, a plant, or any other life form, into a plant. Scientists refer to this process as transformation, which is carried out using laboratory techniques such as (1) particle acceleration, (2) protoplast transformation, and (3) transformation mediated by *Agrobacterium* or possibly by endophytes, and (4) chloroplast transformation. To date, perennial grass plants have been transformed using all these techniques except chloroplast transformation (Mikkelsen et al. 2001; Somleva, Tomaszewski, and Conger 2002; Wang, Hopkins, and Mian 2001). The transferred gene often is termed the gene of interest, meaning the DNA that allows the plant to express the desired trait. *Selectable markers* confer traits that allow recognition of plants that have been transformed; herbicide or antibiotic resistance commonly is used as a selectable marker. There is concern that certain of the select-

able markers could result in harm to ecosystems, agriculture, or human health if they should "escape" to naturalized organisms. Genes of interest and selectable markers, which in certain instances are one and the same, are examples of *transgenes*, or DNA incorporated into the plant using a transformation technique. Transgenes include the gene of interest and/or the selectable marker, plus additional DNA, such as a promoter, that regulates when and where in the plant the gene is active.

Most plant transformation techniques involve tissue culture. This process involves placing plant tissue, such as a germinating seed, on a culture medium that provides nutrients and hormones allowing the production of a *callus*, a group of unorganized plant cells. Under proper media and growth conditions, calli can form embryos and eventually entire plants.

Particle Acceleration

Particle acceleration, also referred to as *biolistics*, involves shooting a gene into plant cells and subsequently growing a plant from the cells containing the transgene. Small gold or tungsten particles are coated with DNA containing the transgene. A device referred to as a gene gun or particle gun uses a propellant such as helium to shoot the particles into plant cells derived from tissue culture (Figure 3.8). Certain of the metal particles enter living cells that in turn can take up the DNA coding for the transgene. The callus tissue is placed on media containing a selection agent such as an antibiotic or herbicide that normally kills plant cells. Plant cells transformed with the selectable marker gene are resistant to the antibiotic or herbicide, continue growing, and eventually can produce a green plant (Figure 3.8). *Whiskers transformation* is a process similar to biolistics, except that plant cells are stirred in a liquid containing silicon carbide fibers coated with the transgene, facilitating insertion of the DNA into plant cells like small needles.

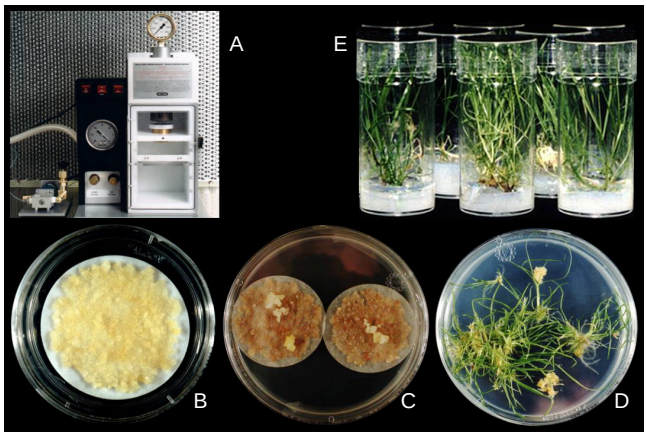

Figure 3.8. **Generation of transgenic tall fescue plants by biolistic transformation. A. Biolistic device used for microprojectile bombardment. B. Suspension cells of tall fescue plated on filter paper before microprojectile bombardment. C. Hygromycin-resistant calli obtained after bombardment of suspension cells with particles coated with a hygromycin resistance gene (*hph*). D. Transgenic plantlets regenerated from the hygromycin-resistant calli. E. Transgenic tall fescue plants (from Wang, Hopkins, and Mian 2001).**

Protoplast Transformation

Protoplasts are living plant cells with the cell walls removed. They are transformed by being placed in liquid with a transgene. An electrical shock (*electroporation*) or the chemical polyethylene glycol (PEG) assists the DNA in entering the plant cell. Tissue culture then is used to generate green plants.

Agrobacterium-mediated Transformation

In nature, the bacterium *Agrobacterium tumefaciens* can transfer a portion of its DNA to a number of plant species. Under certain conditions this common phenomenon causes a disease in woody plants known as crown gall. The genes that cause gall formation are found on a circular fragment of DNA known as a *plasmid*. These genes can be removed from *Agrobacterium* and a transgene for a new trait inserted. These *Agrobacterium* cells then are suspended in a liquid medium, sometimes under vacuum pressure (Trinh et al. 1998) along with plant tissue such as leaf material. During this time the bacterial plasmids transfer the transgene to some of the plant cells. The plant tissue is grown on a special medium to kill any residual bacteria and nontransformed plant cells. Eventually, transgenic plants can be recovered.

Endophyte-mediated Transformation

Naturally occurring fungi living inside plants, known as *endophytes*, are found in several grass species. In many instances endophytes can be dispersed only when the plant produces seed or via vegetative plant parts. Endophytes may prove useful as surrogate organisms for grass transformation; they can be removed from plants and placed in culture. During this time, transgenes can be inserted into the endophyte using some of the same procedures used for plant transformation. The transformed endophyte

then is injected inside grass seedlings free of endophytes. Under proper conditions, seedlings become infected and eventually produce seed containing the transformed endophyte.

Chloroplast Transformation

Photosynthesis takes place within plant cells in small organelles called *chloroplasts*. These same organelles contain DNA, and because they normally are not carried in the pollen, they are maternally inherited and are passed on to offspring only through seed. Chloroplasts are maternally inherited in many perennial grasses (Corriveau and Coleman 1988; Martinez-Reyna et al. 2001), although exceptions may exist (Kiang et al. 1994). Chloroplasts can be transformed using biolistics procedures (Svab, Hajdukiewicz, and Maliga 1990). The spread of transgenes through pollen would not occur for plants with maternally inherited transformed chloroplasts. This also would be true for plants derived from endophyte-mediated transformation. Chloroplast transformation may be useful in obtaining elevated levels of gene expression because multiple copies of chloroplast genes can be highly expressed. Chloroplast transformation has not been reported to date in perennial grasses.

Use of Biotechnology Compared with Conventional Procedures in Cultivar Development

Biotechnology offers the opportunity to impart traits that might not be possible otherwise, to improve existing traits, and to improve understanding of the genetics and biology of perennial grasses. Ordinarily, genes can be transferred only within a species, or between closely related species, using conventional plant breeding procedures. But genes from any living organism can be transferred to a plant using biotechnology. As a result, biotechnology allows plant breeders to impart traits to a given plant species that might not be available otherwise. For example, most perennial grasses are not naturally resistant to herbicides such as glyphosate (trade name Roundup®) or glufosinate (trade name Finale®) that kill a very broad spectrum of weeds. Using biotechnology, scientists can insert genes from bacteria, creating herbicide-resistant plants (Somleva, Tomaszewski, and Conger 2002).

Biotechnology often is used to transfer only one or a few genes (Figure 3.9). This ability to modify specific genes has given scientists a better understanding of the genetics and biology of plants (e.g., Guo et al. 2001; Springer 2000). In conventional plant breeding, transfer of only one gene is very difficult because of genetic linkage, whereby a gene and adjoining DNA often are inherited together (Young and Tanksley 1989). *Linkage drag* results when undesirable DNA is transferred along with the intended gene. Although biotech transformation methods can result in transfer of unwanted DNA to plants (Smith, Kilpatrick, and Whitelam 2001), such occurrences can be identified, and the resulting plants typically are discarded (Mumm and Walters 2001).

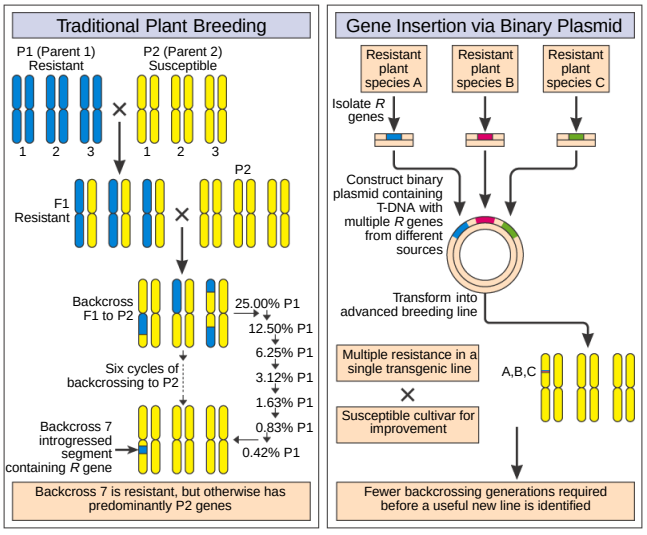

Figure 3.9. Conventional plant breeding methods for introducing one or a few genes require making crosses, usually within the species. Then a series of backcrosses needs to be made to return to an acceptable cultivar. A decrease in breeding time and effort is possible by inserting genes directly into the embryogenic callus of useful genotypes. The technique also would allow genes from very different species to be incorporated into perennial grasses (Adapted from Buchanan, Gruissem, and Jones 2000).

Several challenges exist when using biotechnology for cultivar development. As with conventional breeding, a number of plants must be modified to obtain the desired trait while minimizing any unwanted side effects. Typically, very few plant cells are transformed (Figure 3.10). Of these, only a portion will go on to produce mature plants. In addition, a number of factors can cause unsatisfactory expression of transgenes in plants. Multiple copies of a transgene can be incorporated into chromosomes of the recipient plant, leading to decreased expression of the gene of interest, which is desirable in some but not all cases. Insertion of a partial transgene can lead to limited expression as well. Transgenes can become

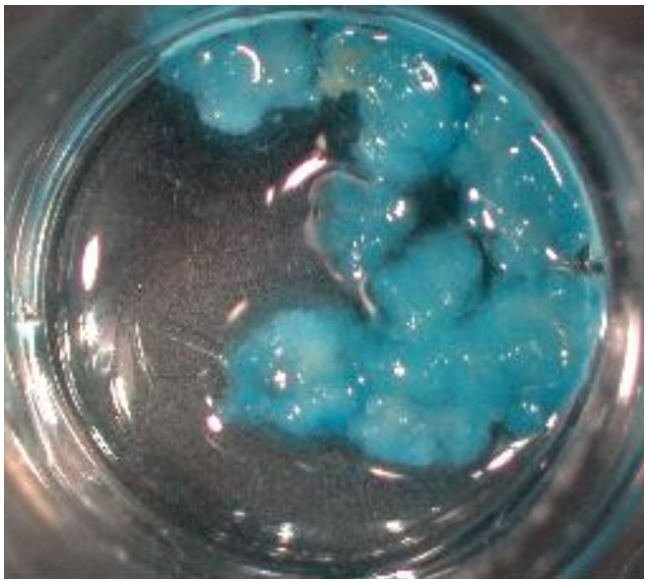

Figure 3.10. The *E. coli* ß glucuronidase (GUS) reporter gene has been used in perennial grasses to test expression of transgenes. Here is an example of a transgenic bermudagrass callus expressing the GUS reporter gene. Photo courtesy of Rongda Qu, North Carolina State University.

inserted within an existing gene in the recipient plant, resulting in altered or lost expression of that gene. Alternatively, insertions into certain sections of DNA result in limited transgene expression. Finally, the transgene can be inserted properly but is not expressed as desired because the plant modifies the translation or the product of the transgene.

The integration of a transgene into a specific location in the DNA of the recipient plant is termed an *event*. Satisfactory events are those that impart only the intended trait, allow the trait to be predictably passed on to subsequent generations, and contain only the desired DNA. A number of events normally are generated, and of these only one or a few events for a given trait are used in cultivar development (Mumm and Walters 2001). Selection of such an event primarily focuses on the healthiest or best-transformed plants similar to the parents or agronomic cultivars with the traits needed for commercial acceptance.

Cultivars of perennial grasses often are a group of genetically different plants. *Inbreeding depression*, whereby plants lose vigor or display other anomalies, can result when closely related perennial grasses are interbred. To avoid this situation, a useful event must be incorporated into a number of plants using conventional breeding procedures. This can result in linkage drag when a substandard plant is used as the recipient for the transgene. Linkage drag can be avoided by using plants from superior populations in the transformation process. The closer the plant to be transformed is to the desired agronomic performance level, the less conventional breeding may be required to develop a useful cultivar.

For many traits incorporated into BD grasses via a transformation event, there still are some uncertainties over the requirements for cultivar composition. It is not always known whether all the plants of a cultivar need to possess the new trait or if a subset of plants will provide sufficient expression in the new cultivar to have the desired effect. Because most perennial forage and turf grasses are polyploid, there also is the question of gene action (*additive* vs. *dominant*) and gene dosage. For example, in an *autotetraploid* grass, there are five possible gene dosages of a transgene (0, 1, 2, 3, or 4 doses). Unless all parents of a cultivar are uniformly homozygous for four doses, the new cultivar will segregate for the transgene, and progeny plants may vary in expression of the transgene. If the transgene is related to plant fitness, this could result in genetic shifts during seed multiplication and after a cultivar is deployed into a turf or forage sod. Because transgenes can be silenced (their expression turned off) and the silencing process is unpredictable, it is not known exactly how plant phenotypes will be affected by transgene segregation or dosage in cultivars of BD grasses (Gallie 1998; Matzke and Matzke 1995).

Finally, effective transformation processes have not been developed for certain important species of perennial grass. In other instances, certain populations of a perennial grass are not easily transformable. This often is because certain populations or species do not perform well in tissue culture. For example, bentgrass is easy to grow in tissue culture and regenerates whole plants without much modification of the documented techniques. Bermudagrass, however, will produce undifferentiated cells in tissue culture quite well, but whole plants are difficult to produce.

Examples of Traits Targeted for Improvement by Biotechnology in Perennial Grasses

Improvements in perennial grasses are focused primarily on reducing both biotic and abiotic stresses that reduce the productivity of these grasses or require additional inputs such as water, fertilizers, and pesticides. The use of biotechnology currently is being investigated for several specific improve-

ments; a few examples are outlined briefly in this section. To date, however, there are no deregulated BD perennial grasses available for commercial production.

Improved Plant Stress Tolerance and Productivity

Improved stress tolerance is expected to have several results: decreased need for inputs such as pesticides and irrigation water for turfgrasses; drought tolerance and improved productivity of forage grasses; and enhanced persistence of perennial grasses in general. Stress can be classified broadly as *biotic*, that which is caused by a living organism (e.g., fungi, bacteria, viruses, insects, etc.), and *abiotic*, that which is caused by factors other than living organisms (e.g., heat, drought, salt, cold, etc.).

Resistance to Plant Pathogens

Fungal cell walls contain chitin, a compound that is degraded by the enzyme chitinase. Grass plants genetically engineered to produce a chitinase, using a gene from an elm tree, have shown increased resistance to a disease caused by a fungus (Chai et al. 2002). Pokeweed Antiviral Protein (PAP) was found to decrease the occurrence of turfgrass diseases and was considered a candidate for transgenic grasses (Dai et al. 2003). Even though both chitinase and PAP were introduced successfully into perennial grasses, pathogen resistance was not considered high enough to proceed with commercialization (Figure 3.11). Bacterio-opsin and glucose oxidase also have been examined as candidate transgenes in perennial grasses (Belanger et al. 2000). But much more needs to be understood about how these disease-resistance genes will work in an entirely different species. Also, it would be desirable to introduce a single gene that produces long-lasting disease resistance because grasses are perennials. The use of several genes may confer the long-term resistance needed for perennial grasses.

Herbicide Resistance

Weeds encroach into perennial grass areas, and the introduction of a nonselective herbicide to reduce weeds would be desirable. This is true especially if there are no herbicides currently available to control the undesirable weed species that invade turf and forage grasses. Scientists have developed both glyphosate- and glufosinate-resistant perennial grasses successfully using biotechnology (Lee et al. 1995, 1996; Lee 1996). Currently, glyphosate-resistant creeping bentgrass (*Agrostis stolonifera L.*) is under consideration for deregulation by the USDA–APHIS.

Coat Protein Genes

Viruses often are not a major problem in perennial grasses; however, for instances such as ryegrass mosaic virus, they can reduce forage yield. Virus pathogens often change the host plant's physiology to benefit the growth and replication of the virus; this change can result in decreased plant performance or death. On entering a plant, a virus must remove its *coat protein*(s) in order to replicate itself and thus cause disease symptoms. Plants able to produce coat proteins of a specific virus can be resistant to disease caused by that virus. A gene coding for a coat protein of ryegrass mosaic virus was introduced into perennial ryegrass and showed promise for conferring resistance to that virus (Xu, Schubert, and Altpeter 2001).

Insect Resistance

Naturally occurring fungal endophytes already are available in turf species such as ryegrass, tall fescue, and the fine fescues, but host plant and fungal interactions are very complex and need more investigation (Richardson, White, and Belanger 1998). Endophytes produce alkaloid compounds in the plant and can impart resistance to insect pests. Certain of these alkaloid compounds, however, are undesirable in forages because they can decrease animal performance.

Figure 3.11. Interesting transgenes that have demonstrated resistance to plant pathogens have been tested in perennial grasses. Research with pokeweed antiviral protein demonstrated that certain transgenes may transfer or increase disease resistance. This particular resistance, however, only delayed disease onset by 2 weeks.

One early approach to biological control was to inoculate endophyte-free turf species with a promising endophyte in the hopes of establishing insect or disease resistance. Unfortunately, it is not easy to move the existing endophytes from species to species. More basic research is needed to capitalize on this existing biological control method.

Other microorganisms found in the *rhizosphere* have a productive symbiotic relationship with perennial grass roots or leaves. Transformation of these species to produce antibiotics to ward off disease or insect problems may be possible someday and could help decrease the need for pesticides. These organisms also might be transformed to include the N-fixing genes of *Rhizobium* to provide N for perennial grasses and eliminate the need for N fertilizers.

Drought Tolerance

Decreasing the quantity and quality of water required by perennial grasses would help decrease demands on potable water in certain regions of the United States. The mannitol 1-phosphate dehydrogenase (*mt1D*) gene was inserted successfully into creeping bentgrass (*Agrostis palustris* Huds. cv. Penncross) in an effort to improve drought tolerance (Redwine 2000; Redwine, Baird, and Sticklen 1999). The *mt1D* gene is reported to aid in drought and salinity tolerance via *osmoregulation* through the production of mannitol. Mannitol accumulation in tissues of regenerated clones and nontransformed bentgrass was evaluated under salt and drought conditions in greenhouse studies; however, there were no significant differences reported in response to either stress. These results suggest that single transgene insertion to solve complicated abiotic stress problems may not be an effective technique.

Regulatory elements induce expression of a number of genes in response to a stimulus, such as drought or cold. Research is under way to modify genes encoding for regulatory elements, such as DREB (dehydration responsive element-binding protein) and CBF (C-repeat binding factor)-like genes, in order to increase the drought tolerance of plants. This also may result in improved tolerance to cold and salt stress (Kasuga et al. 1999).

Regulation of Senescence

Grass leaves normally *senesce*, or become yellow, as they age. So-called stay-green genes prevent senescence and have led to increased stress tolerance and productivity of other crops. Decreased senescence could lead to decreased protein breakdown in forage grass leaves, and thus improved protein utilization by grazing livestock. Increased retention of greenness also could result in more attractive turf.

Fructan Accumulation

Many grasses accumulate the carbohydrate fructan. The ability to accumulate fructan may lead to improved tolerance to drought (Pilon-Smits et al. 1995) and cold stress (Thorsteinsson, Harrison, and Chatterton 2002), and to enhanced forage quality. Bacterial genes have been introduced into perennial ryegrass that allow the production of a form of fructan that may be accumulated in much larger quantities than normally are found in plants; however, growth of these plants was affected negatively (Ye et al. 2001).

Improved Phosphate Uptake

The element phosphorus (P) is essential to growth and development of both plants and animals. Phosphorus is bound tightly in soil and can be difficult for plants to take up in sufficient quantities. Phosphorus fertilization can result in improved productivity of forages (Gelderman, Gerwing, and Twidwell 2002) and decrease the risk of *grass tetany* (Lock et al. 2002), a serious health problem that sometimes causes death in livestock. When applied at high rates, however, P fertilizer can be expensive and increases the risk of P pollution in runoff water. Enzymes such as acid phosphatase solubilize forms of P that otherwise would be unavailable to plants. In an attempt to improve the ability of plants to take up P from the soil, a gene coding for acid phosphatase has been identified and is being linked to promoters that will allow expression specifically in roots.

Apomixis

Apomixis is asexual reproduction through seed. An apomictic plant produces progeny that are genetically identical to itself. This means that a superior plant can be propagated through seed, in contrast to cultivars of perennial grass that often consist of a genetically variable population of plants. Introduction of apomixis through biotechnology may allow genetically superior individuals to be propagated via seed in forage grasses where this trait currently is not available. One possible drawback to using apomixis is that genetic uniformity may be associated with a lack of resistance to a disease or insect pest in a plant population.

Improved Animal Health and Productivity

Perennial forage grasses are the foundation for the production of ruminant livestock (e.g., dairy and beef cattle, sheep, goats). Forage quality is the extent to which a forage, whether pasture, hay, or silage, has the ability to produce the desired animal response. While many factors affect forage quality, the stage of maturity at harvest is the most important consideration. Protein content, digestibility, and acceptability to livestock drop as grasses move from the vegetative, or leafy, stage to the reproductive, or seed, stage. For instance, grasses may contain more than 30% protein at the immature, leafy stage, but drop to less than 8% protein when they mature (Heath, Metcalfe, and Barnes 1973).

In addition, plant species is an important factor in that considerable variation in quality exists among the various forage species. Generally, cool-season grasses usually are more digestible than warm-season grasses. Plant breeders continue to improve forage quality within species, so variation also exists within species among varieties. Improved forage quality often is a major goal when developing perennial grass cultivars through conventional breeding or biotechnology for forage applications. Use of these cultivars by farmers and ranchers will result in improved animal health and productivity.

Regulation of Flowering

Except for seed production, it rarely is desirable for perennial grass to flower and produce stems and seed heads. In turf applications, flowering increases mowing requirements and decreases turf quality. Stems almost always have lower forage quality than leaves, and can cause eye irritation to grazing livestock. Flowering perennial grasses also produce pollen that in certain instances can cause allergic reactions in humans. Finally, flowering can lead to *ergot*, a fungal disease of seeds that results in toxin production and can cause health problems when consumed by grazing animals. Researchers are using biotechnology or conventional means in an attempt to alter genes that control flowering in perennial grasses so that flowering can be eliminated or greatly decreased, such as in forage and turf applications, or induced when needed, such as in seed-production fields. If successful, this system could result in effective genetic containment, in that transgene movement through pollen or seed would be unlikely if not impossible, except perhaps in seed-production fields.

Manipulation of Lignin Biosynthesis

Lignin, a class of compounds found in plant cell walls, is important in providing structural support and disease resistance to plants. Lignin interferes with the digestion of plant cell walls and essentially is indigestible itself. Transgenic plants of tall fescue, an important perennial grass, have been produced with altered genes coding for the enzymes CAD (cinnamyl alcohol dehydrogenase) and COMT (caffeic acid O-methyl transferase), which are involved in lignin production (Chen et al. 2003). In certain plants, altered composition and decreased concentration of lignin resulted in substantially increased forage digestibility. The tall fescue genes coding for lignin synthesis were *down-regulated*, meaning they had decreased expression. Down-regulation can be accomplished by *sense suppression*, whereby multiple copies of a gene are introduced into plant chromosomes, or by *antisense suppression*, whereby a transgene is arranged in the opposite direction of the *endogenous* gene.

Manipulation of Pollen Allergens

It has been estimated that 20% of the human population in temperate areas of the world has an allergic reaction to proteins found in grass pollen (Wang, Hopkins, and Mian 2001). In ryegrasses, which are important turf and forage grasses, transgenic plants have been generated that produce pollen with decreased allergenicity; pollen from these plants was otherwise normal (Bhalla, Swoboda, and Singh 1999). This same approach should be possible with other allergy-causing grasses.

Increased Sulfur Concentration in Plant Proteins

Wool production in sheep often is limited by the amount of sulfur (S) absorbed by the animal's digestive system. This condition may be caused by limited S concentrations in forage and the inefficient digestion of proteins in the rumen. Tall fescue plants have been transformed with a gene from sunflower that codes for a protein rich in S (Wang et al. 2001). This protein seems to be resistant to breakdown in the rumen, and thus should be absorbed more efficiently in the lower digestive tract of sheep. If successful, this approach may lead to more efficient wool production and may have application in improving protein use in other ruminant animals.

4 Gene Migration and Weed Management of Biotechnology-derived, Perennial Grasses

Biotechnology-derived (BD), perennial grasses raise concerns for weediness and invasiveness because certain aspects of their biology may increase the potential for gene flow and persistence. Two major concerns expressed about BD perennial grasses are that the inserted gene will migrate into related species, and that the BD grass or hybrids with related species will become invasive weed problems in agricultural and natural landscapes. In this section, these concerns are discussed in three subsections: "Gene Migration," "Herbicide Resistance," and "Invasiveness." Gene migration deals with the means by which the inserted gene may move into related species via pollen, seed, or vegetative propagules. Herbicide resistance is dealt with specifically because scientific information from other crops and perennial grasses exists for this particular trait and directly impacts weed management. Invasiveness is discussed because of concern about the weed management of BD perennial grasses bred for herbicide resistance or increased fitness.

Gene Migration

Gene migration, which can occur through pollen, seed, or vegetative propagule movement, is an important consideration in the risk assessment performed by regulators for BD plants. Species that are wind pollinated with a high rate of *outcrossing* can disperse their seed efficiently, have prolonged seed longevity, can persist outside of cultivation, and have sympatric wild or *weedy* relatives with which to cross are more likely to spread their genes. Many perennial grasses possess some or many of these characteristics. The impact of an introduced trait that may increase, decrease, or have a neutral effect on gene migration and a plant's fitness and competitive ability will need to be considered on a case-by-case basis, however.

Although perennial grasses represent a multitude of species with diverse growth habits, life histories, and reproductive biology, these are important considerations for all BD plants; as discussed earlier, information addressing these characteristics is required by regulatory agencies such as the U.S. Department of Agriculture (USDA) in assessing the plant pest risk of a BD plant. For example, cotton, canola, rice, and sugar beet (Johnson and Burtch 1958; McFarlane 1975) exist outside cultivated areas and have feral or weedy relatives. Representative USDA risk assessments have been completed for all these BD crops (USDA–APHIS 1995, 1998a, 1999a, b). Guidance documents that identify the types of data required by the USDA and the Canadian Food Inspection Agency (CFIA) also were published (CFIA 2002; CFIA, USDA–APHIS, USEPA 2000; USDA–APHIS 1998b, c). In the case of cotton, the U. S. Environmental Protection Agency (EPA) did not permit the production of BD plants with a pest-resistant trait in areas that have wild or weedy relatives.

Pollen Movement

Gene migration via pollen movement will be influenced by (1) the level of outcrossing that occurs in the species, (2) the occurrence of sympatric compatible relatives, and (3) whether their flowering times overlap. If hybrids occur, do they persist, produce viable seeds, and act as bridges to related species?

Predicting gene migration with pollen dispersal is difficult. In pollen dispersal studies, pollen often is found at the boundary of the study so that the only accurate conclusion is that pollen moved that particular distance. Extrapolating beyond the boundaries of the experiment based on modeling the data can lead to inaccurate conclusions about pollen dispersal. For example, Wipff and Fricker (2001) attempted to model gene flow from creeping bentgrass. The outcrossing frequencies were extremely low at the furthest collection point (less than 0.01%). In addition, the r^2 values supporting the Wipff and Fricker model were very low, indicating that the predictions may be unreliable. As already discussed, careful attention must be paid to research results and subsequent extrapolation when used beyond the boundaries of the experiment. The experimental outcrossing frequencies with related species and the

distance from parent plants reported by Belanger et al. (2003), Christoffer (2003), and Wipff and Fricker (2001) are summarized in Table 4.1.

Pollen dispersal models of the perennial grass species *Lolium perenne* have been developed. Giddings, Sackville Hamilton, and Hayward (1997a) tested pollen dispersal models suggested by Bateman (1947). Pollen traps were used to predict pollen dispersal, but whereas pollen grains were counted, their viability was not tested. One conclusion of the researchers was that there was high variability of pollen deposition and further refinements of the equations were needed. Giddings, Sackville Hamilton, and Hayward (1997b) analyzed pollen dispersal, and taking into account wind direction with distance, they concluded that in addition to wind speed and direction, turbulence also should be considered.

Giddings (2000) also used a model to predict the outcome of pollen dispersal from a large-scale planting into a small *conspecific* population. Although pollen dispersal was predicted to move a kilometer from the source and swamp the small population, this was not confirmed in the field. Measuring or predicting pollen dispersal without measuring pollen viability, hybridization, seed production, and viability of the hybrids is of little value. Techniques must be developed to measure pollen viability over time and distance in addition to pollen movement.

Gene flow from hybridization of cultivated plants into wild relatives (*introgression*) has been well documented for many plants (Ellstrand, Prentice, and Hancock 1999). In at least one instance, gene flow from conventionally developed sorghum (*Sorghum bicolor*) was determined to have increased the fitness of the weed Johnsongrass (*S. halepense*) (Snow and Palma 1997). Many of the naturalized perennial grasses have wild relatives in the United States (Hitchcock 1951).

There have been reports of *interspecific crossing* within the genus *Agrostis* by several authors (Belanger et al. 2003; Bradshaw 1958a; Davies 1953; Jones 1956a, b, c; Tutin 1980; Wipff and Fricker 2001). In the studies conducted by Bradshaw (1958b), Davies (1953), Jones (1956a, b, c), and Tutin (1980), however, these hybrids were produced artificially and typically were of intermediate morphology between the two parents. In addition, they had either a complete loss or reduced fertility. Intergeneric hybrids also have been reported between *A. stolonifera* and *Polypogon* spp., but the hybrids were sterile (Björkman 1960).

Recent studies have evaluated outcrossing and hybridization of BD *Agrostis stolonifera* with non-BD *A. stolonifera* and related *Agrostis* spp. (Belanger et al. 2003; Christoffer 2003; Wipff and Fricker 2001). Wipff and Fricker reported that in 1998 outcrossing occurred at very low frequencies at 91 meters (m)—the limit of the study—from the source plants. In 1999 they expanded the limits of the study to 300 m and still found outcrossing at the limits of the study. The limits of the Belanger et al. (2003) study were only 15 m, and hybrids were identified at 15 m. Christoffer (2003) also found intraspecific outcrossing to the limits of the study at 345 m, but neither interspecific nor intergeneric outcrossings were found at distances greater than 50 m. In all these outcrossing and hybridization studies, seed were formed on many but not all of the related *Agrostis* spp. Differences occurred among the studies as to

Table 4.1. Percentage of gene flow by distance as reported in the scientific literature

Species	Gene flow[a] (%)	Distance in feet (meters)	Reference
Canola	0.6	1,200 (365.76)	Bing (1991)
Smooth bromegrass	0.2	640 (195.07)	Knowles (1966)
Bromegrass	0.6	960 (292.61)	Jones and Newell (1946)
Crested wheatgrass	3.0	400 (121.92)	Jones and Newell (1946)
Intermediate wheatgrass	3.0	400 (121.92)	Jones and Newell (1946)
Buffalograss	6.3	640 (195.07)	Jones and Newell (1946)
Switchgrass	0.4	960 (292.61)	Jones and Newell (1946)
Rye	0.6	1,280 (390.14)	Jones and Newell (1946)
Corn	0.5	960 (292.61)	Jones and Newell (1946)
Perennial rye	0.8	60 (18.29)	Griffiths (1951)
Italian rye	0.8	60 (18.29)	Griffiths (1951)
Creeping bentgrass	0.06	1,200 (365.76)	Christoffer (2003)
Spring wheat	0.004	23 (7.01)	Hucl and Matus-Cadiz (2001)
Rice	0.01	16 (4.88)	Messeguer (2003)

[a]Jones and Newell (1946) measured pollen flow rather than gene flow. Pollen viability, however, was evaluated and deemed sufficient to pollinate receptive plants at the distances for each species evaluated.

which crosses were successful. In addition to *Agrostis* spp., Christoffer included *Polypogon* spp. and found seed was produced by interspecific crosses with *A. stolonifera*.

Hybrids between grasses are reported in the literature. Bradshaw (1958a) reported F_1 hybrids between *Agrostis tenuis* Sibth (*A. capillaris* L.) and *A. stolonifera* were more frequent than either parent in a pasture, and they were better adapted to grazing conditions than either parent. The author suggested that the hybrid was more competitive with other grasses and with its parents, and that the hybrids were able to occupy different habitats than either parent, thereby having a greater ecological range. The hybrids had low fertility but were not 100% sterile. Nonetheless, Bradshaw (1958a) suggested that sterile or partly sterile vegetative hybrid clones would spread and persist only in conditions of high disturbance and low environmental stress, and that the perennial success of a colony would decline during periods when these conditions were not met, suggesting that the long-term survivability of sterile clones is limited in nature. The ability of the hybrid to act as a bridge with either parent was not determined. Bradshaw noted further that it might be difficult to identify hybrids because their form would reflect the form of the parents, which could vary from site to site.

Hybrids are reported between *Lolium* spp. and *Fescue* spp. (Berg, Webster, and Jauhar 1979), Kentucky bluegrass and other bluegrasses (Wedin and Huff 1996), and bermudagrass and related *Cynodon* spp. (de Wet and Harlan 1970; de Wet, Harlan, and Richardson 1969). Although hybrids for these perennial grasses are known to occur, their impact on ecosystems is unknown.

To date, no studies on the impact of backcrosses from introgression events in perennial grasses have been published. There is concern about this because backcrosses have occurred in other BD crops. For example, backcrosses of transgenic, interspecific hybrids of canola (*Brassica napus*) and field mustard (*B. rapa*) resulted in plants that were essentially *B. rapa* with herbicide and insect resistance genes (Chrispeels and Sadava 2003). Ultimately, it has to be assumed that gene flow will occur. Yet the need to conduct such studies must be examined on a case-by-case basis that considers the species and gene in question. Unless the particular trait confers a selective advantage or provides improved fitness of wild populations, such studies would be unlikely to provide new information on invasiveness. Moreover, these are long-term studies that would be difficult to conduct during the time frame allowed to deregulate BD perennial grasses.

If hybrids are produced, they may be less fit, equally fit, or more fit to either parent. Hybrids may be fully fertile, have reduced fertility, or be sterile. The likelihood of outcrossing with related species also would depend on the geographic distribution for the related species that are known to cross.

Genes that cause a plant to become more fit than its non-BD varieties or wild relatives could conceivably enhance its potential to become an invasive weed. Factors that may enhance fitness include superior tolerance to moisture, light, wind, and temperature extremes; enhanced seed production; and/or increased vegetative spread—all factors that breeders would like to add to existing varieties and are evaluated by the USDA in their risk assessment of BD plants. Snow and colleagues (2002) showed that the *Bacillus thuringiensis (Bt)* gene placed into cultivated sunflowers (*Helianthus annuus*) could transfer into wild sunflowers (also *H. annuus*); the progeny had increased seed set and fewer pests. Increased seed set, however, would be considered an advantage for a plant species. It is unknown if such a genetic transformation would translate to enhanced fitness.

It is possible that expression of a new gene has a metabolic cost or could decrease fitness in other areas; however, to date there is little evidence to support this among BD plants that have been commercialized. In fact, metabolic or fitness costs associated with the transgene likely would impact plant growth and yield, which would decrease their acceptance in the marketplace. To the contrary, adoption of BD crops has increased annually since their introduction in the mid-1990s (USDA–ERS 2003).

In the sunflower project (Snow et al. 2002), a greenhouse test indicated the transgene did not provide any cost or benefit in the absence of insect pests. If a pest or environmental variable is the limiting factor for plant spread, removal of the barrier may not necessarily enhance the plant's fitness. For example, leafspot disease (caused by *Drechslera* and *Bipolaris* spp.) was the limiting disease for Kentucky bluegrass use before the release of resistant varieties in the mid-twentieth century. By the 1970s, a disease eventually diagnosed as necrotic ring spot caused by *Leptosphaeria korrae* became the limiting disease for Kentucky bluegrass use in turf.

Another consideration is that conventional breeders have tried for centuries to breed plants that have various characteristics. Specifically, grass breeders have bred grasses with superior tolerance to environmental stresses such as drought, temperature, and light. In certain cases, grasses have been bred for im-

proved vegetative spreading ability, seed yield, or traffic tolerance. These examples provide familiarity with BD perennial grasses modified to express similar traits.

Seed Movement

Seeds are dispersed in space and time. Seed dispersal in space is related to how far a seed will move, whereas dispersal in time is related to the length of seed viability and dormancy. The argument often is made that perennial grasses are not allowed to go to seed on well-managed golf courses and playing fields, so gene migration is not likely to occur. This argument ignores the fact that certain golf courses and playing fields are not well managed. In addition, perennial grass seeds tend to be small and therefore can be moved easily by natural means. Seed size among the perennial grasses varies from extremely small for bentgrass (0.07 mg per seed) to fairly large for perennial ryegrass (1.9 mg per seed). Even the largest perennial grass seed is much smaller than soybean or corn. Wind, water, and animals disperse seeds, and it is not possible to control these natural dispersal agents.

Unlike BD annual crops that already have been introduced, perennial grasses have a longer and more indeterminate seed life in the soil. Rampton and Ching (1970) buried seed of several grasses and exhumed them at varying time intervals for 7 years. They reported that after 7 years of burial, annual ryegrass had 0.2% germination, whereas perennial ryegrass lived only 4 years. No live orchardgrass, tall fescue, or Chewings fescue seeds were found 3 years after burial. Highland bentgrass was the most persistent grass seed with 1.8% germination after 7 years. Rampton and Ching (1970) also reported differences among cultivars of the species tested. Although the percentage of germination was low, viable seeds remained in the soil for several years. Therefore, seed life in the soil should be considered when deregulating BD perennial grasses.

Federal certification requirements for the production of Foundation, Registered, and Certified seed can be found at <http://www.ams.usda.gov/lsg/seed.htm>. In Oregon, where a significant amount of grass seed production is located, the usual requirement for certification states that 5 years must elapse between planting crops of the same species. These requirements are needed because of soil seed longevity that could lead to crop contamination.

Vegetative Propagule Movement

Gene migration by vegetative propagules needs to be considered with perennial grasses because many grasses reproduce from stolons, rhizomes, or roots (Table 3.6). But available data are lacking on survival of vegetative propagules under different environmental conditions. These data, however, are required by the USDA to assess whether BD plants are more or less likely to persist than their non-BD counterparts (USDA 2002). In addition to containing a viable *meristem* (*node*), the size requirement for a propagule to produce a daughter plant is unknown. Also unreported is the level of desiccation, cold tolerance, or heat tolerance that a propagule can withstand and still remain viable. Small vegetative propagules can be moved in the same way that seeds are moved.

Seed production is not necessary for a plant that reproduces vegetatively. For example, *Arundo donax* L. is an invasive perennial grass in California. Its spread is due entirely to fragmented stem and rhizome pieces because it does not produce viable seed (Boose and Holt 1999).

As with seeking deregulation of all BD grasses from the USDA, careful attention to potential migration via pollen, seed, and vegetative propagules of the BD perennial grasses is required. Pollen movement cannot be prevented in outcrossing, wind-pollinated species, and seed movement will occur during seed production and marketing. Finally, the movement of vegetative propagules also is likely to occur. The question that must be addressed, however, is not whether gene migration will occur, which is likely with all BD crops, but the frequency with which it occurs (which is dependent on the species and trait) and its impact on the environment.

With respect to perennial grasses, forage grasses allowed to pollinate may result in considerably faster gene flow to related species than intensively managed bentgrasses maintained on golf courses at heights below which they will pollinate. Therefore, questions related to the potential environmental hazard(s) associated with gene flow or the impact that gene migration will have on plant populations in managed and unmanaged areas and the likelihood or frequency of its occurrence should be considered. Although gene flow may be an economic issue in crop production or marketing, these issues are not pertinent to an environmental risk assessment and will not be addressed in that context here.

Herbicide Resistance

Herbicide resistance has been defined as the ability of a formerly susceptible plant population to survive herbicide doses above those that once were used to control the original plant population (Ross and Lembi 1999). This can be contrasted to *herbicide tolerance,* which has been defined as the ability of a plant population to remain uninjured by herbicide doses normally used to control other plant species. Herbicide tolerance also refers to the concentration of herbicide residue allowed in or on agricultural products (Ross and Lembi 1999). Therefore, the practical difference between herbicide resistance and herbicide tolerance is that the former refers to a species that once was controlled by a herbicide and no longer is controlled, whereas the latter refers to a species that never was controlled by a specific herbicide. Herbicide-resistant populations develop as a result of selection pressure placed on a plant population by the continued use of the same herbicide or herbicide with the same mode of action. Powles and Holtum (1994) described two precursors for the evolution of herbicide resistance in plant populations: the occurrence of (1) heritable variation for the trait and (2) natural selection. Further, the evolution of resistance under persistent applications of a herbicide may be considered as an example of recurrent selection.

Herbicide resistance is not likely to provide an advantage in environments in which the herbicide is not used and indeed may be a disadvantage (Belanger et al. 2003; Bergelson et al. 1996; Dyer et al. 1993; Johnson and Riordan 1999; Meagher, Belanger, and Day 2003; Quemada 1999). Herbicides are used widely in a range of environments, however, and over large areas, so selection for hybrids containing a herbicide-resistant gene could occur. If plant species invasive in natural or agricultural areas acquired herbicide resistance, it could make their control more difficult if no other herbicides were available. This rarely is the case because several effective herbicides are available for perennial grass control in both of these settings that could be used to control a herbicide-tolerant grass (*Crop Protection Reference* 2003). Adaptive traits such as drought resistance, salt tolerance, and cold or heat tolerance could increase the ecological range of the species. These traits could provide an advantage for survival and also could allow certain species to occur in environments where they previously could not survive.

Once a population evolves resistance to a particular herbicide, it is likely that it will be resistant to other, closely related herbicides. *Cross-resistance* is a specific term used to describe weed populations that are resistant to two or more herbicides that have the same mode of action (Ross and Lembi 1999). For example, in certain instances the herbicides may be in the same chemical family (plants resistant to simazine also are resistant to atrazine even though they never have been exposed to atrazine), or they may be in different families that have the same mode of action (plants resistant to dinitroaniline herbicides also may be resistant to dithiopyr, which belongs to the pyridine family but has the same mode of action as dinitroanilines). *Multiple resistance* is used to describe weed populations that are resistant to two or more herbicides from different chemical families and with different modes of action (Ross and Lembi 1999). Fortunately, multiple resistance occurs infrequently, whereas cross-resistance tends to be more common.

The development of herbicide-resistant weed populations is an increasing problem in worldwide agricultural systems. The Weed Science Society of America (WSSA), in conjunction with the Herbicide Resistance Action Committee and the North American Herbicide Resistance Action Committee, reports 275 herbicide-resistant biotypes (see WSSA website at <http://www.weedscience.org/in.asp>). They report 79 biotypes resistant to herbicides that inhibit acetolactate synthase (ALS inhibitors), 64 biotypes resistant to herbicides that inhibit photosynthesis at photosystem II (such as triazine herbicides), and four biotypes resistant to glyphosate, which inhibits EPSP (5-enolpyruvylshikimate-3-phosphate) synthase.

Johnson and Riordan (1999) discussed potential risks and benefits of transgenic turfgrasses. Regarding the development of herbicide-resistant turfgrasses, one of the risks identified by the authors was the tendency for turfgrass managers to become more dependent on the herbicide. This dependence on a particular herbicide or herbicide mode of action would increase selection for herbicide-resistant weeds in wild populations. If transgenic creeping bentgrass resistant to a herbicide is available to turfgrass managers, a major target weed will be *Poa annua* L. (Johnson and Riordan 1999). Johnson (1995) described *P. annua* as an extremely variable species, and McElroy (2002) suggested that such variation can be attributed to ecological adaptation to habitat. *Poa annua* also has a large soil seed bank (Lush 1988), a fast generation time (Johnson 1995), and gene flow between populations (Wu, Till-Bottraud, and Torres 1987). These characteristics are of con-

cern in evaluating the possibility of herbicide resistance development in wild populations of *P. annua*.

Research is under way to develop BD grasses that use a gene for herbicide resistance. In situations in which herbicides are not used, a gene for herbicide resistance is not likely to confer a competitive advantage, and escapes or *interspecific hybridization* events are not likely to become invasive (Belanger et al. 2003; Bergelson et al. 1996; Dyer et al. 1993; Johnson and Riordan 1999; Meagher, Belanger, and Day 2003; Quemada 1999). Of greater concern may be the unintended contamination by herbicide-resistant grasses in areas where the herbicide is used to control unwanted vegetation. Herbicides often are used to control unwanted vegetation in managed turf and forage sites as well as in natural areas. Means to limit the occurrence of herbicide-resistant grasses in undesirable areas range from disallowing use of the gene to management practices that would greatly diminish gene flow (or plant movement) to other options (e.g., different herbicides). In all likelihood, certain escapes will occur at some point in time. There also is potential for the gene(s) to migrate to relatives. Lastly, increased herbicide use could lead to development of herbicide-resistant weeds. Certain weed populations such as those in velvetleaf (*Abutilon theophrasti*) have either inherent or acquired tolerance to glyphosate (Hartzler and Battles 2001). Other weed populations seem to have developed resistance in the presence of areas treated repeatedly with glyphosate (e.g., horseweed [*Conyza canadensis*]; Van Gessel 2001).

Development of herbicide tolerance may not increase the fitness of a weed to survive and spread, however, as was demonstrated in the instance of glyphosate-tolerant velvetleaf (*Abutilon theophrasti*) (Hartzler and Battles 2001). Velvetleaf, a broadleaf weed common in Midwestern agronomic crop fields, has developed glyphosate tolerance, although its inherent fitness is not superior to glyphosate-sensitive types (Hartzler and Battles 2001). Grasses also may develop resistance to glyphosate through repeated exposure without the occurrence of gene movement from transgenic plants (Goss et al. 2001; Powles, Lorraine-Colwill, and Preston 1998). Glyphosate is a very popular nonselective herbicide used throughout the United States in agronomic, turf, and natural settings, and its loss of activity would require herbicide users and crop managers to use other herbicides or alter their practices.

Herbicide-resistant weeds have developed in the absence of biotechnology, and given the widespread use of glyphosate, it is possible that glyphosate-resistant weeds will occur even without development of glyphosate-resistant BD plants. But by increasing the use of glyphosate over more acres, the chance of glyphosate-resistant weeds occurring would tend to increase. Herbicide resistance in weed populations has been noted for other herbicides, however, and users have adopted different herbicide or cultural management strategies. In addition, breeders have been developing glyphosate-tolerant grasses using conventional breeding strategies based on naturally occurring tolerance (Johnston and McBride 1990), and two varieties of fescue with tolerance to glyphosate have been commercialized by Turf Seed, Inc. These varieties have been available commercially for several years without documented evidence of weed resistance despite the lack of a recommended weed resistance management plan.

Glyphosate is a highly effective, nonselective herbicide that is foliar-applied to emerged weeds (Vencill 2002). Herbicidal activity first was reported in 1971. Selected formulations can be used in BD crops tolerant to glyphosate such as soybean, corn, cotton, and canola (Vencill 2002). Glyphosate is absorbed across the cuticle and is translocated primarily in the *symplast* with accumulation in underground tissues, immature leaves, and meristems (Vencill 2002). Glyphosate toxicity in plants is a result of inhibition of ESPS synthase, which produces EPSP from shikimate-3-phosphate and phosphoenolpyruvate in the shikimic acid pathway. Such EPSP inhibition leads to depletion of the aromatic amino acids tryptophan, tyrosine, and phenylalanine, all which are needed for protein synthesis or for biosynthetic pathways leading to growth.

The WSSA reports six species of weeds with resistance to glyphosate: horseweed (*Conyza canadensis*) in the United States, hairy fleabane (*Conyza bonariensis*) and buckhorn plantain *(Plantago lanceolata*) in South Africa, goosegrass (*Eleusine indica*) in Malaysia, Italian ryegrass (*Lolium multiflorum*) in Chile, and rigid ryegrass (*L. rigidum*) in Australia (see WSSA website at <http://www.weedscience.org/in.asp>). In addition, all have been reported in the last 10 years. In the instance of glyphosate-resistant horseweed in the United States, six cases have been reported, and the first case was found in Delaware in 2000 (Van Gessel 2001). Mueller (2003) described the accumulation of shikimate in glyphosate-sensitive and glyphosate-resistant horseweed populations. Measurement of shikimic acid accumulation in response to glyphosate inhibition of EPSP synthase is a rapid and accurate *assay* to quantify glyphosate injury in sensitive plants (Mueller 2003). Shikimate

concentrations in all untreated horseweed plants were significantly less than in plants treated with glyphosate. In treated plants, however, shikimate accumulated in both the resistant population and the susceptible population. There were differences in shikimate accumulation patterns between resistant and susceptible horseweed biotypes. Shikimate concentrations in resistant populations declined approximately 40% from 2 to 4 days after glyphosate treatment, whereas shikimate concentrations in the susceptible horseweed plants increased about 35% from 2 to 4 days after treatment. The authors further suggested that the shikimate accumulation data indicate that the mechanism of glyphosate resistance in horseweed is not due solely to a single, glyphosate-insensitive EPSP synthase, because if a glyphosate-resistant EPSP synthase were present, significant increases in shikimate would not be expected.

Mueller (2003) also reported that resistant horseweed plants could tolerate four times the normal application dosage of glyphosate but exhibited certain herbicidal effects from the herbicide, such as yellowing in actively growing apical meristems. The four instances of weeds developing resistance to glyphosate reflect significantly fewer than the 79 biotypes resistant to ALS-inhibiting herbicides and 64 biotypes resistant to herbicides that inhibit photosynthesis at photosystem II. But a side-by-side comparison of the potential for a single species to develop resistance to these herbicides has not been performed until the recently published work of Jander et al. (2003). In their study, the frequencies of resistance of glyphosate and two ALS-inhibiting herbicides—chlorsulfuron and imazethapyr—were evaluated in a controlled ethylmethansulfonate (EMS) saturation *mutagenesis* experiment.

This experiment (Jander et al. 2003) allowed a direct comparison of the frequencies at which resistant mutants to glyphosate, chlorsulfuron, and imazethapyr herbicides arise. The 100% growth inhibition dose rate of these herbicides was determined for *Arabidopsis*. On the basis of data available at the time of the study, it was calculated that a population of 125,000 EMS-mutagenized lines is needed to have a 95% chance of finding a mutation in any given base pair that can be mutated by EMS (Haughn and Somerville 1987). Two 125,000-plant populations of EMS-mutagenized *Arabidopsis* lines were sprayed with twice the 100% growth-inhibition dose of glyphosate, chlorsulfuron, and imazethapyr, and herbicide-resistant mutants were identified. No glyphosate-resistant seedlings were identified among the 250,000 seedlings evaluated, whereas chlorsulfuron and imazethapyr mutations each appeared at frequencies of 3.2×10^{-5}. The researchers concluded that no single-base change induced by EMS could produce glyphosate resistance.

Because only six species of weeds have developed resistance to glyphosate in the past 25 years despite its widespread use, and the work of Jander et al. (2003) suggests that the frequency of development and survival of glyphosate-resistant mutants is extremely small, it is not likely that the release of BD perennial grasses will dramatically increase the number of glyphosate-resistant populations. Given that other weeds have developed resistance to glyphosate, however, and that certain problematic weeds in turf such as *Poa annua* L. are highly variable species, there exists the possibility that resistant weed populations may occur. Therefore, if BD perennial grasses with resistance to glyphosate are released, it is suggested that companies producing such varieties provide guidance to users that prevents the onset of resistance. Such proven strategies include, but are not limited to, rotation of herbicide modes of action.

Invasiveness

Most of the dozen or so turfgrasses and certain of the forage grasses used in the United States originated on other continents and were introduced after European colonization (Casler and Duncan 2003; Huff 2003). Kentucky bluegrass (*Poa pratensis* L.), the most widely used cool-season turfgrass, probably was introduced into the United States in the seventeenth century (McCarty 2001). Perennial ryegrass (*Lolium perenne* L.), used both as a forage and a turfgrass, is native to southern Europe, western Asia, and northern Africa (McCarty 2001). Tall fescue (*Festuca arundinacea* Schreb.), used originally for forage and additionally for turf since the latter part of the twentieth century, is native to the Mediterranean region (Sleper and West 1996). Zoysiagrasses (*Zoysia* spp.) were introduced from Southeast Asia in the early twentieth century, whereas bermudagrasses (*Cynodon* spp.), native to Africa, were introduced about 1750 (McCarty 2001). Nonnative turfgrasses are desirable because most native grasses are not capable of forming medium- to high-quality turfs. In many instances these grasses have naturalized and may be found in unmanaged sites (Levine 2000). The nativity of some of the grasses is unknown or debated by scientists (Huff 2003). Development of molecular markers and other biotechnology tools may aid

scientists in tracing the origin and evolution of grasses.

Invasiveness is defined in ecological terms as "the ability of a species to naturalize and spread in places where it is not native." In most instances invasiveness also requires economic or environmental harm to be caused before the term is applicable (Baskin 2002). Invasiveness usually is discussed in terms of natural environments. In natural settings, invasive species can crowd out or eliminate native species, causing a decrease in biodiversity and possibly affecting wildlife and the environment. Most of the land in the United States has been disturbed by human activity in the past (Cowell and Jackson 2002; Hobbs 2000), however, and urban and many rural areas cannot be considered natural because they are managed actively by humans. Nonetheless, in the instance of agricultural and landscaped areas, certain species may be considered invasive if they affect productivity (forages) or other use (turf) adversely, requiring specific and sometimes costly management practices.

Several groups have or are developing invasive species lists; for example, the National Invasive Species Council (2003), the Plant Conservation Alliance (2003), and The Nature Conservancy (2003). A number of perennial grasses appear on these lists including many commonly used for turf and forage such as Kentucky bluegrass and tall fescue. At least one organization, the Invasive Plants Association of Wisconsin (IPAW), is developing a tiered approach to identify the degree to which species are invasive and their impact (IPAW 2003).

Unfortunately, scientific literature that documents the invasiveness of perennial grass species is scarce. Levine (2000) observed the presence of *Agrostis stolonifera* and other nonnative plants in a riparian community dominated by *tussocks* of sedge (*Carex nudata*). When *A. stolonifera* seed was planted in sedge tussocks supporting various numbers of other indigenous species, *A. stolonifera* establishment success was proportional inversely to the number of indigenous species existing within the tussocks of sedge. Establishment success at 3 weeks after spring seeding was similar to success by early autumn. Pammenter, Drennan, and Smith (1986) noted *A. stolonifera* was recognized first on the wind-swept sub-Antarctic Marion Island in 1965 and had become abundant in wet, protected, coastal stream areas by 1981. It had longer internodes than the indigenous *A. magellanica* Lam., shorter leaf life, and slightly superior carbon assimilation at low photon flux densities. The lack of strong shoots in *A. stolonifera* apparently confined it to wind-sheltered areas, whereas the stronger shoots of *A. magellanica* enabled it to persist in exposed sites. Floras and unpublished monitoring data may help document introduced or invasive species; ideally, such information would show an increase in area covered by a species over time before being listed as invasive.

Predicting Invasiveness

The invasiveness of BD perennial grasses will depend on three factors: (1) the inherent invasiveness of the plant species, (2) the type of gene(s) introduced, and (3) the environment. A viable model to predict invasiveness of perennial grasses does not exist, primarily because of limited funding to support research and the complexity of modeling the invasiveness of plants. More than 30 years ago Baker (1965, 1974) developed a list of characteristics for determining invasive weeds: good competitors in their native range; long leaf lives; polyploid genomes; and successful reproductive strategies, either from seed, vegetative propagules, or both. Many invasive weeds also would have a short juvenile period and an extended length of flowering; be self-compatible and/or use unspecialized pollination mechanisms (e.g., wind); and have effective seed-dispersal mechanisms, a range of seed dormancies, and high seedling vigor. Anecdotal evidence, however, indicates certain invasive plants do not require these characteristics, whereas many noninvasive plants do have one or more of these characteristics (Baskin 2002).

The invasiveness of BD grasses should not be equated to the invasiveness of exotic introductions. Although most introduced plants are not invasive in their new habitat, those that are invasive typically are good colonizers at their site of origin (Reichard and Hamilton 1997) and possess many weed traits. Biotechnology-derived grasses typically will vary in only one or a few genes compared with their progenitors, theoretically making it easier to predict their invasiveness compared with exotic introductions (Hancock and Hokanson 2001). In general, cultivated grasses are weak competitors in unmanaged environments (Quemada 1999). Most turfgrasses require mowing, fertilization, and irrigation to persist in a monostand. Of course, any grass can be considered a weed if it appears in a situation where it is not wanted (e.g., creeping bentgrass in a Kentucky bluegrass lawn, Kentucky bluegrass or smooth brome in range land, etc.).

Species Basis

Predicting the invasiveness of a BD perennial grass may be accomplished partly through familiarity (i.e., assessing the inherent invasiveness of the wild-type). A thorough prediction is complex and complicated by the paucity of scientific evidence for invasiveness of most perennial grasses. The role of growth habit on the invasiveness of grass species, for example, is not well established. Certain species such as tall fescue rely on seed for distribution, whereas others such as bermudagrass often are established by vegetative propagules such as rhizomes or stolons. Additional information may be found in the section "Vegetative Properties."

Biotechnology-derived plants are not likely to produce results any different from results for plants developed using conventional breeding methods. The USDA has deregulated 61 BD plants (all of which contained genes from unrelated organisms) after coming to a "Finding of No Significant Impact," which would suggest that transformation impacts the plant in a predictable manner (i.e., they express the phenotype resulting from the introduced trait). A number of scientists and scientific bodies have come to the same conclusion. For example, the National Academy of Sciences (NAS–NRC 1989) concluded that crops modified by genetic engineering should pose risks no different from those of crops modified by classical genetic methods (including bridging crosses, wide crosses, mutagenesis, etc.) for similar traits and grown in similar environments. The NAS reiterated this position in its 2002 report *Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation*, which stated repeatedly and considered as a foundation of its evaluation the fact that biotech plants present no unique risks compared with crops developed through conventional means (NAS–NRC 2002).

Effect of Gene Type

There is a lack of scientific data in the public domain on how the addition of specific genes will affect the invasiveness of perennial grasses. Crawley and colleagues (2001) monitored four annual crop plants, sown in natural areas, for 10 years for BD traits including herbicide and insect resistance. They concluded that the BD lines had similar or less persistence than their non-BD counterparts. This does not mean, however, that traits for environmental stress tolerance or pest resistance identified in the section "Specific Traits Targeted for Improvement by Biotech in Perennial Grasses" automatically can be determined to have no environmental impact. As described in the same section, however, biotechnology will allow the introduction of specific genes rather than a plethora of genes that may occur through conventional breeding. Thus the transformed plant may act in a more predictable manner, similar to a nontransformed counterpart, with the exception of the trait(s) conferred by the added gene(s) (Hancock and Hokanson 2001). This will allow evaluation for environmental impact to be conducted on a targeted aspect(s) of the BD plant. Certain gene insertions actually may reduce potential for invasiveness by reducing fecundity, as described in the section "Regulation of Flowering." Ultimately each gene type will have to be evaluated on a case-by-case basis.

The Environment

The potential for any plant to become invasive depends largely on the environment. The environment includes both *biotic* and *abiotic factors* that may influence the potential for invasiveness. Abiotic factors may include, but are not limited to, temperature, moisture, soil, and light. An example of wind limiting the colonization of a sub-Antarctic island by *Agrostis stolonifera* already has been described (Pammenter, Drennan, and Smith 1986). Changes in the environment can help a plant become invasive. For example, cheatgrass (*Bromus secalinus*) became invasive in Canyonlands National Park in southern Utah after a particularly rainy winter in 1984–1985, which facilitated establishment of seeds that had been blowing in from outlying areas (Baskin 2002). Unfortunately, it is nearly impossible to predict environmental changes.

Biotic factors (animals, plants, and microbes) also may affect invasiveness. For instance, Kentucky bluegrass may dominate pastures that have been overgrazed by cattle and other species that are encouraged by the additional fertility supplied by manure and that can withstand the frequent grazing. In most instances, however, it is difficult to determine the effect other *biota* may have on the invasive potential of a species. Scientific data vary on the effects of native plant diversity to resist weed invasions; ultimately, biodiversity may not affect an ecosystem's ability to resist invasive species (Levine and D'Antonio 1999). Most difficult to understand is how microbes (e.g., bacteria, fungi, nematodes) may influence the success or failure of a given plant species.

Microbial populations are tremendously diverse and difficult to study. Many microbes cannot be isolated and grown in sterile (*axenic)* culture. Microbes that can be cultured require specific parameters that preclude isolation of other microbes. But specific studies should not be performed unless there is reason to suspect the trait will impact microbial populations.

5 Criteria for Evaluating Biotechnology-derived, Perennial Grasses

An important function of this publication is to provide guidance to government agencies responsible for the determination of how to regulate biotechnology-derived (BD) plants. This chapter provides a general discussion of the criteria to be considered for the unconfined release of BD grasses. Issues related to the seed, flowering, and vegetative properties of BD perennial grasses were examined during the workshop. An extensive list of questions was developed to help direct the discussion by the invited speakers and meeting attendees during the breakout sessions held during the workshop (see Chapter 6). Table 5.1 contains additional websites that provide valuable information on other Animal and Plant Health Inspection Service (APHIS) documents that deal with BD plants.

This chapter provides a summary of the key issues for each of the three categories: (1) seed properties, (2) flowering properties, and (3) vegetative properties. This is by no means, however, a comprehensive review of all possible questions and issues raised at the workshop or submitted in written comments. The authors have tried to provide a perspective, based on their professional and scientific expertise, of issues needing the most emphasis when considering the unconfined release of BD perennial grasses.

Table 5.1. Websites with information concerning the deregulation of biotechnology-derived plants

The APHIS has produced a User's Guide for Petitions (<http://www.aphis.usda.gov/ppq/biotech/petguide.html>).
The APHIS and the Canadian Food Inspection Agency (CFIA) (<http://www.inspection.gc.ca/english/plaveg/pbo/dir/dir0007e.shtml#1.2.8>) have summarized the molecular genetic characterization data for transgenic plants intended for unconfined release in Appendix I (http://www.inspection.gc.ca/english/plaveg/pbo/appenannex1e.shtml>). The APHIS uses Appendix I as the basis for its review of the molecular biology of the plants being considered for deregulation.
The APHIS has prepared a list of characteristics it identifi es as central to the reproductive and survival biology of plants. The Office of Science Technology and Policy first published this list for public comment in January 2001 in Case Study 3 (<http://www.ostp.gov/html/012201.html>).
In conjunction with the CFIA, the APHIS used this list of characteristics to develop the "Environmental Characterization Data for Transgenic Plants Intended for Unconfined Release" (Appendix II) (<http://www.inspection.gc.ca/english/plaveg/pbo/appenannex2e.shtml>). This is the most comprehensive list of the questions that applicants need to address in a petition for deregulation. But because the biology of the plant, the phenotype of the plant, and the environment of introduction are different for each petition, additional questions always are required.
The APHIS has provided more specific guidance on the types of data to collect for the annual row crops, corn and cotton (<http://www.aphis.usda.gov/ppq/biotech/cornguid.html> and <http://www.aphis.usda.gov/ppq/biotech/Cotton_ag.html>).

Seed Properties

Issues for seed properties of BD perennial grasses vary to a certain degree as they relate to commercial seed production, plant-breeding objectives, and maintained turfgrass conditions. These issues and concerns also vary between cool- and warm-season grasses, and the many genera, species, and cultivars that comprise the perennial grasses. With this in mind, it is impossible to make categorical recommendations across all perennial grasses. Primary concerns for seed production of BD perennial grasses include pollen movement and viability, seed dispersal and movement, and seed contamination.

Commercial Seed Production

Commercial seed production of perennial grasses has several requirements: appropriate climatic conditions; intense management; and adequate infrastructure to support producers and production concerns, meet regulatory requirements, condition seed, and manage the end product. Therefore, commercial seed production often is concentrated in fairly specific geographic regions. Good examples of concentrated seed production are the cool-season turfgrasses produced in the Pacific Northwest (Cowan 1969).

Pollen Movement and Viability

With concentrated production, there is greater opportunity for pollen movement from one seed production field to another compared with sites where production is dispersed widely. Regulatory require-

ments dictate isolation distances between experimental fields, where pollen movement and contamination may be an issue (Bateman 1947; Cowan 1969; Griffiths 1951). Even with isolation, pollen will move from field to field, particularly in areas of concentrated production (Belanger et al. 2003; Bradshaw 1958a). Pollen movement and potential contamination may not be discerned easily. In the instance of BD perennial grasses, however, such as herbicide-tolerant types, recognizing the degree of pollen movement is much easier. Pollen drift and *outcrossing* can be identified readily by herbicide selection (Christoffer 2003). Research using this approach has demonstrated that the distance grass pollen travels is relatively consistent, decreasing rapidly with distance from the source. Jones and Newell (1946) confirmed the efficacy of distance isolation by studying the distribution of pollen at various distances from source populations of several grass species. They found that the average amount of pollen captured at 300 meters (m) (990 feet [ft]) from the source was <1% of that found at the source; however, there was no attempt to measure the viability of the pollen. They attributed the rapid decline in pollen concentration to gravity and dispersion.

Knowles (1966) reported an outcrossing frequency of 0.2% at 195 m (640 ft) with smooth bromegrass. More recently, Christoffer (2003) reported intraspecific creeping bentgrass pollen to travel as far as 365 m (1,200 ft) in both 2001 and 2002 but with a hybridization frequency of approximately 0.05%. Wipff and Fricker (2001) also measured bentgrass pollen that traveled up to 292 m (958 feet) from the source during 1999, but only a single transgenic hybrid was identified among more than 1,000 evaluated (0.001%).

Even though research demonstrates the potential for movement, it does not substantiate that pollen traveling long distances will be viable or capable of competing with the existing pollen load among adjacent plants within a field. Pollen-mediated gene flow experiments conducted with isolated receptor plants will have a tendency to overestimate the amount of pollen movement with *intervarietal* crossing under production field conditions, however, because isolated or small populations of pollen receptor plants are more apt to be pollinated by their nearest neighbor than a remote pollen source (Copeland and Hardin 1970; Griffiths 1951; Knowles 1966). The factors that will impact cross-fertilization between grasses include synchrony of pollen shed and receptive stigmas (date and time of day), proximity of the plants, wind speed and direction, pollen viability and longevity, temperature, relative humidity, and compatibility between pollen and stigmas or styles (Allard 1960; Burton 1992). Size of the plant population and planting density also have major impacts on outcrossing frequency because of *intravarietal* (within a single field) pollen competition for receptive stigmas.

In a series of pollination studies designed to examine isolation distance and competing pollen sources in perennial ryegrass, Griffiths (1951) concluded that the effects of intravarietal pollen competition were highly effective in reducing intervarietal crossing (between fields) and more effective than distance because the nearest neighboring plants are likely to provide the biggest pollen contribution. Similar conclusions were drawn by Heribert-Nilsson as cited in Griffiths (1951) for rye, by Knowles (1966) for smooth bromegrass, and in Copeland and Hardin (1970) for perennial ryegrass. These results help provide the basis for Griffiths' recommendation for (and the currently common practice of) cutting border rows from large Certified fields after pollination where adequate isolation cannot be provided by distance.

Pollen movement in seed production is an issue for both BD and traditionally derived, cross-pollinated species. But the level of concern is dependent entirely on the species, traits being expressed, and the potential for outcrossing to wild-types or field-to-field contamination. As previously mentioned, it may be easier to determine the BD perennial grass pollen movement compared with the pollen movement of traditionally derived perennial grasses, making gene flow more readily assessed in seed production. An assessment of pollen movement, viability, and fertilization frequency for BD perennial grasses in comparison with their nontransgenic counterparts will assist in the basic understanding of whether differences between these varieties would be expected. If differences are detected, these findings may lead to a reconsideration of the standards for isolation distances. The potential for contamination of native populations is limited somewhat in turf and forage perennial grasses. Most of these species, with the exception of a few warm-season species, were introduced and are not native to North America. Many have native *congeners* with which they are known to hybridize (Table 3.1). Wild relatives, however, even though not natives, still are important in considering gene movement because many are unwanted invasive species in natural areas.

Hybrid formation between BD grasses and *conspecific weedy* or *wild* relatives is dependent largely on the ability of the crop to deliver pollen to the recipient. In turn, this is influenced heavily by the degree

of *sympatry* shared by the crop and its recipient in terms of both their geographical and ecological distribution and on the strength of mechanisms favoring inbreeding over outcrossing (Wilkinson 2002). Nonetheless, for many crops, pollen-mediated gene flow from crop to feral populations is of little importance because it is evident that such populations are both ephemeral and rare. For these plants to become invasive, the transgene would need to confer a significant selective advantage (Wilkinson 2002).

Seed Dispersal and Movement

Significant effort is spent in maintaining the genetic purity of commercially produced turf and forage grass seed lots (Cowan 1969). These efforts involve seed production, harvest, conditioning, packaging, and distribution. Even though efforts are made to avoid contamination from seed dispersal and movement, there are numerous opportunities for contamination to occur. In production fields, wind, water, and animals can disperse seed. During harvest, combines require thorough cleaning to minimize the potential of contamination as the units move to different cultivars, and from field to field. The seeds of most perennial grasses are quite small, making cleaning no easy task. Cleaning certainly is a critical step during the seed-conditioning phase, and care must be taken to avoid contamination during storage before conditioning and after conditioning, and before bagging and packaging the final product. These are important concerns for both BD and traditionally derived perennial grasses.

Biotechnology-derived grass seed dispersal and movement is of particular concern after packaging, during storage or transport, and when the end user handles seed. Biotechnology-derived perennial grass seed will require stringent guidelines and oversight during these times to minimize the potential for seed movement into areas where the BD grasses are not desired. There simply is no way to ensure that no seed will be lost or dispersed during any of the stages—production, harvest, transport from the field to seed conditioning, packaging, transport to market, or end-user application. Thus it is critical to assess risks adequately before making a decision on deregulation.

Plant Breeding and Maintained Turf Situations

Plant breeders face similar issues to those involved in the commercial seed production of BD perennial grasses. Seed production in maintained turf is not likely to be a major concern. Essentially there is no viable seed production in turfs that receive weekly or more frequent mowing (Beard 1973). Low-maintenance turf sites such as roadsides or turf areas that are mowed only once or twice a year may produce viable seed, depending on the frequency and timing of mowing. Even under infrequent mowing, however, there are many confounding factors that make seedling establishment unlikely in a mature *sward* (Watschke and Schmidt 1992), but it would be desirable to use BD perennial grasses under mowed conditions where they are mowed often enough to prevent seed production. Similar approaches may be necessary to minimize the potential for seed production from BD perennial forage grasses. There always is a potential for seed production in pasture and forage sites, particularly around the field margins and in fencerows.

Issues and Concerns for Risk Assessment

Many issues and concerns relating to BD perennial grass seed production will vary on a case-by-case basis because of differences in species and cultivar pollination biology, length of flowering season, self-compatibility, and degrees of *inter-* and *intraspecific crossing*.

Risk assessment for BD perennial grass seed production will need to take into account:

1. Seed viability, dormancy, and transport;
2. Seedling emergence, vigor, and survival;
3. Testing locations within and outside the normal range of seed production for the species; and
4. Data collection from well-established stands, mowed versus unmowed areas, spaced versus solid row planting, and maintained turf versus seed production conditions.

The Plant Variety Protection Act 1970 (PVPA) and the National Variety Review Board have addressed certain of these issues and concerns (Stanton 1997). For example, using two locations in one test year rather than testing over 2 years at the same location may give a better indication of seed production potential because of the greater environmental variation included in the design. Publications such as *Crop Science* and the *Agronomy Journal* expect a minimum of 2 years or two locations for most field-oriented research. It also is understood that more locations and more years of testing increase precision and would be desirable as situations allow.

Seed production testing for BD perennial grasses should be done for normal seed production, and pro-

duction conditions should be similar to those used for commercial seed production for the species. Seed production characteristics are determined best under conditions that foster competition rather than individual, spaced plantings. Certain warm-season species require a closed canopy to evaluate seed yield potential. It is understood that production practices and conditions vary between warm- and cool-season species.

Flowering Properties

Forage grass seed is used primarily in the central and southern United States, whereas turfgrass seed is used throughout the United States on golf courses, home lawns, athletic fields, roadsides, and sod farms.

Flowering Types

In plants, seed production results from the fertilization of a female egg cell by a male pollen cell, allowing genetic exchange, or recombination, to occur between plants. Alternatively, plants can produce seed asexually by *apomixis,* whereby recombination does not occur.

Perennial grasses have evolved a number of mechanisms for producing seed. Many grass plants are *monoecious*, meaning that a single plant contains both male and female floral organs. Individual flowers that contain both male and female organs are said to be perfect. A *dioecious* species produces separate male and female plants; *staminate plants* produce male flowers, whereas *pistillate plants* produce female flowers. *Male-sterile plants* are incapable of producing functional pollen, and thus function as pistillate plants. The majority of perennial grass species are *cross-pollinated*; meaning that pollen from one plant fertilizes the egg of a different plant. Self-pollination results when a plant's pollen fertilizes its own egg cell. Certain grasses are capable of producing flowers and seed both above- as well as belowground (Call and Spoonts 1989).

With minor exceptions (Adams, Perkins, and Estes 1981), perennial grasses rely on wind, rather than insects, to disperse pollen. It is not clear how far grass pollen can disperse and remain viable. Pollen movement is discussed in great detail elsewhere in this publication, but in general, pollination tends to occur to the greatest extent between closely adjacent plants (Copeland and Hardin 1970; Johnson, Bradley, and Knowles 1996; Rognli, Nilsson, and Nurminiemi 2000).

Flowering and pollination of grasses often occur over a period of time within a given season, with ranges greater than 40 days possible within a given cultivar (Boonman 1978). *Indeterminate* plants are capable of flowering multiple times during a growing season. *Determinate plants* will have only one flowering period, and thus produce only one seed crop, per growing season. This often is the case for cool-season grasses with a strong *vernalization* requirement, meaning that plants must be exposed to cool-season temperatures and/or short day-lengths to allow flowering (Heichel, Hovin, and Henjum 1980; Heide 1984).

Criteria for Measuring Flowering Characteristics

All grass cultivars should meet certain standards of uniformity, stability, and distinctness before being placed on the market. In the certification process, information is obtained by the organization releasing a given cultivar regarding growth and appearance characteristics of the plant population, such as vegetative, flowering, and seed traits. Certifying agencies use these cultivar descriptions and additional information, such as field history and location, to determine whether seed produced from a given field meets specific standards of purity. In this way, purchasers of certain seed classes, such as foundation or certified seed, are assured of cultivar identity and purity.

Plant variety protection, or PVP, allows the owner of a cultivar to place certain restrictions on the breeding, production, and marketing of that cultivar. As with the certification process, the PVP Office of the U.S. Department of Agriculture (USDA) grants PVP certificates based on specific information provided by the applicant (see Appendix A or <http://www.ams.usda.gov/science/pvpo/PVPindex.htm>). In this process, a number of plant characteristics are measured and compared for both the new cultivar as well as several standard or control cultivars.

Certain flowering traits are quantitative rather than qualitative. The PVP Office requires that when quantitative characters are used to differentiate a cultivar, the applicant must present "Evidence that tests were conducted in two or more localities or during two or more growing seasons" (see Appendix A). This requirement is to ensure that quantitative data reflect adequately the range in variation for the cultivar, and that these traits are characterized sufficiently. The literature suggests that certain flowering characteristics in perennial grasses, such as heading date (Casler 2001; Casler et al. 2000; Lamb,

Vogel, and Reece 1984), can be characterized adequately in two environments.

Certification and PVP processes do not determine the weediness potential of a cultivar. It is anticipated that these protocols would be useful in establishing guidelines for evaluating the flowering characteristics of BD perennial grasses. Additional research is needed, however, to verify that the traits evaluated and the procedures used during certification and PVP processes are useful in determining the weediness and invasiveness of BD perennial grasses derived from biotechnology.

Vegetative Properties

Many perennial cool- and warm-season grasses can produce vegetative propagules such as *stolons* and/or *rhizomes;* examples are listed in Table 3.6. Many species can be used either for turf or forage.

Vegetative structures that live from season to season provide a means of spread for many perennial grasses. Mother plants produce stolons and/or rhizomes (*perennating organs*) that grow along the surface or belowground, respectively. Rhizomes of certain species (e.g., certain *Cynodon* spp.) may emerge from belowground to form stolons and then reenter the soil and reform rhizomes (Taliaferro 2002). Daughter plants are produced from *nodes* (*axillary buds*) on the stolons or rhizomes. Openings in turf canopies may be important to provide light and space for triggering the development of a daughter plant from a stolon or rhizome node. Stolons and rhizomes may be determinate (single node and daughter plant) or indeterminate (multiple nodes and daughter plants). Grasses that can spread by vegetative structures are said to have creeping growth habits.

Certain grasses, such as hybrid bermudagrass (*C. dactylon* x *C. transvaalensis* Burtt-Davy), are sexually sterile and only can be propagated by vegetative structures (Duble 1996). Most zoysiagrass and St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze) cultivars (1) do not produce sufficient viable seed, (2) produce no viable seed at all, or (3) the seed viability is so poor they cannot be established successfully by seed. Other turfgrasses, such as centipedegrass (*Eremochloa ophiuroides* [Munro.] Hack.), can be established by seed, but because establishment is slow, the preferred form is by vegetative means.

Stolons commonly are preferred most for establishment because they are easier to harvest and produce sods more quickly than rhizomes. Sod can be established from stolons by *stolonizing* or by *sprigging*. In stolonizing, stolons are spread on the ground and may be covered with a thin layer of mulch or soil to encourage their establishment. Sprigging, used for bermudagrass and a few zoysiagrass cultivars, provides a better chance of successful establishment because sprigs (stolon pieces) are pushed into the soil, improving the potential for individual nodes to develop new shoots and roots. *Plugging* is a generic term that refers to planting of a vegetative section (plug) of a stoloniferous and/or rhizomatous turfgrass. Plugs can be grown in trays or can be cut from established turfgrasses and planted. Plugging commonly is used for many zoysiagrass, St. Augustinegrass, and centipedegrass cultivars. Subsequent stolon and/or rhizome growth results in successful establishment.

Unintentional spread of vegetatively propagated grasses can occur when stolons are harvested from a desirable grass sward contaminated with undesirable grasses. Perennial grasses also can be spread unintentionally through transport of soil or equipment infested with stolons or rhizomes.

Impact of Vegetative Spread

Clearly, perennial BD grasses have the potential to spread and become established. For many, vegetative propagation likely will be the preferred method of establishment. The impact of inadvertent vegetative spread of BD grasses will depend largely on the trait and species.

For BD grasses transformed with a gene not known to significantly increase fitness compared with currently available cultivars, the impact of vegetative spread can begin to be addressed by examining the weed problems caused by the species in agronomic and horticultural crops, turfgrass seed and sod production, and natural ecosystems. Centipedegrass, St. Augustinegrass, and zoysiagrass generally are not weeds in agronomic and horticultural crops. In sod production systems where these species are grown on the same farms, however, one species can contaminate another. Bermudagrass is a common weed in agronomic and horticultural crops and sod and seed-production fields for turfgrasses, natural areas across the southern third of the United States, and in lawns and gardens. For several BD agronomic crops such as cotton and soybean, glyphosate commonly is used to remove bermudagrass from infested fields. There may be other chemical controls, both selective and nonselective, for removing BD grasses from undesirable sites (e.g., glufosinate, diquat, fluazifop, imazaquin, and certain sulfonyl ureas). Effective *translo-*

cation of systemic herbicides to stolons and rhizomes is required to contain the bermudagrass. Because bermudagrass is an important weed, extreme care must be taken to ensure that there are adequate control strategies before BD forms are tested. This is more important for bermudagrass than for centipedegrass, zoysiagrass, and St. Augustinegrass, not only because it is a more common weed, but also because it is much more difficult to control.

Determining Vegetative Spread

Many warm-season grasses easily are propagated vegetatively; therefore, tests for vegetative spread should be more inclusive than for BD grasses that do not spread vegetatively. For example, BD bermudagrass should be tested more rigorously than perennial ryegrass.

Bermudagrass, zoysiagrass, and colonial bentgrass produce both rhizomes and stolons, whereas buffalograss, centipedegrass, creeping bentgrass, rough bluegrass, and St. Augustinegrass produce only stolons. Kentucky bluegrass, creeping red fescue, and smooth bromegrass produce rhizomes but not stolons. Bermudagrass, colonial bentgrass, Kentucky bluegrass, and smooth brome may be serious pests in natural areas. The testing procedures used for risk assessment will need to be specific to each BD grass species and should be determined by the method and ease of vegetative spread. The rate of spread from vegetative organs typically will be inversely proportional to the number of competing *tillers*, or density, and aspects related to spread may be predicted in as few as 35 days (Cattani, Miller, and Smith 1996).

The fitness of BD grasses is important in determining if they have increased potential to become weeds. For vegetatively propagated BD grasses, both rhizomes and/or stolons should be measured to determine fitness from estimates of biomass production, leaf width, leaf length, plant height, internode length, shoot density, root biomass, and stolon and/or rhizome production.

The life span of perennating organs should be considered when designing tests for vegetative spread of BD grasses. The life span of these structures will be influenced by the environment. The inherent life span for the perennating organs of a species may or may not be reported in the scientific literature. Therefore, the life span of perennating organs may have to be estimated for certain species. Exact life spans cannot be determined because of environmental influences (e.g., pH, temperature, moisture). These need to be considered in the geographic region(s) where the perennial grass will be grown. In addition, different biotypes of perennial weeds may have different life spans (Lemieux, Cloutier, and Leroux 1993). It will be impractical to conduct research on the survival of all existing biotypes of a species primarily because of the difficulty of identifying all of them. It is possible, however, to evaluate a range of biotypes and to estimate the life span of the plant organs in question. If there are questions concerning increased winter hardiness or heat stress tolerance of a BD grass, then testing procedures should include methods to quantify their effects. These procedures may include field and/or controlled environment studies.

Time Periods for Assessment

The period of time needed to assess the vegetative spread of BD grasses should follow guidelines similar to those for the publication of scientific research that address the variability and characteristics of the grass. For example, *Crop Science* and the *Agronomy Journal* expect a minimum of 2 years or two locations for most field-oriented research. Publication guidelines in these journals do not necessarily reflect ecologically adequate requirements, however, which usually are more than two sites, or 2 years, because of environmental variability. It also is understood that more locations and more years of testing increase precision and would be desirable as situations allow. Analysis of the statistical power of experimental design should accompany results, especially when results are not significant. Where increased stress tolerance is an issue, testing at more than one location outside of the normal geographic areas should be required. Whereas the time period for survival of vegetative propagules will depend on the species, the size and hardiness of the organ and the environment will affect the survival and development of new plants. Lemieux, Cloutier, and Leroux (1993) reported that rhizome meristems of quackgrass (*Elytrigia repens*) died within 2 years under no-till and plowed field conditions, except for one biotype that survived at low levels for a maximum of 30 months. Multiple locations over the geographical area where the BD grass could be expected to survive would enhance the knowledge base.

Site Types for Assessment

Perennial grasses can thrive in managed and nonmanaged environments. Plant vigor can vary wide-

ly depending on interspecific and intraspecific competition. Kendrick and Danneberger (2002) showed that seedlings of creeping bentgrass could not establish in a mowed putting green. Conversely, *Poa annua* plants frequently establish themselves from seed on closely mowed putting greens. Plants developing from vegetative structures typically will benefit from the greater energy availability compared with that available to a seedling, enhancing the likelihood of successful establishment (Howe and Snaydon 1986). Therefore, an accurate picture of vegetative vigor can be determined only by quantitative data over a range of sites. Vegetative vigor should be evaluated in managed and nonmanaged sites as well as with spaced plants growing in bare soil. Planting should be carried out under optimal conditions to establish a benchmark of maximum survival potential (Howe and Snaydon 1986).

Symbiotic Organisms

Grass *symbionts* are microbes that coexist on or in grasses and may influence their behavior. In certain instances their existence is desirable, in others it is not, and in many situations their presence and/or effects are relatively unknown. Traits introduced using biotechnology may impact the presence or effect of symbionts. To date, no published research has documented efforts to use biotechnology to transform grass symbionts. Nonbiotechnological efforts have been undertaken to develop or enhance symbiotic relationships in grasses, and such a system is a likely target for biotechnology, particularly with regard to endophytes.

Although BD plants are not likely to affect soil biota directly, Snow and Palma (1997) suggested that leachate from BD plants could alter the activity of soil organisms. They suggested that toxicological studies should be conducted to ascertain the impact of BD plants on the overall activity of soil biota, rather than on individual soil organisms that may have a negligible impact on the ecosystem itself because of overlapping functions of soil biota. Pesticides may provide the closest analogy to BD grass effects on soil biota, even though pesticides often are intended to affect microbes and other biota directly. Historically, pesticides based on heavy metals often did have long-term effects on soil biota, decreasing populations of earthworms and other biota. Such pesticides now are banned in the United States. The current classes of pesticides do not seem to have a long-term negative impact on soil biota, inasmuch as nontarget microbial populations are able to ignore or degrade the pesticides, with certain pesticides experiencing enhanced biodegradation after repeat applications (Fulthorpe, Rhodes, and Tiedje 1996; Nicholson and Hirsch 1998; Niemczyk and Chapman 1987). Targeted pest populations also may develop resistance to the pesticides designed to inhibit their activity, indicating the resilience of microbial populations to synthetic compounds (Burpee 1997; Vincelli and Dixon 2002). *Auxin-type pesticides* have been shown to improve colonization and nitrogen fixation of microbes (Yu, Kennedy, and Tchan 1993).

Endophytes

Endophytes are fungi that grow within grass crowns and shoots in a symbiotic relationship. Endophytes obtain nutrients from their host while conferring several growth advantages compared with noninfected plants. The primary advantage to plants is decreased herbivory because of endophytic production of alkaloid toxins, which make plant tissues unpalatable or toxic to insects and other herbivores. Although desirable in a turf situation, endophytic grasses may be a liability in forage areas because the toxins may injure livestock. Fungicide seed treatments may be used to eradicate endophytes (Siegel et al. 1984), and postemergent fungicides also may quell endophytes populations. Endophytes, however, may produce secondary beneficial effects on their hosts such as enhanced disease resistance and heat or drought tolerance (Arachevaleta et al. 1989; West 1994). Endophytes also increase phenotypic variation and may allow mixed populations of endophytic and nonendophytic plants to extend their range (Hill et al. 1990). Certain grass genera such as *Festuca* and *Lolium* often are endophytic, although the proportion of endophytic plants may range from 0 to nearly 100% depending on the cultivar, the endophyte, and seed storage conditions. Infection is rare or limited in other genera such as *Poa*.

The majority of *endophytic mycelium* exists in the basal portions of shoots and crowns; thus these tissues are most useful for determining the presence of endophytes in plants. Endophytic detection in seed also is possible (Hill et al. 2002). Microscopic examination of plant tissue has been used to determine the presence of endophytes. Recently, simple immunological detection kits have been produced for certain types of endophytes, particularly those in *Festuca* and *Lolium* (Hill et al. 2002).

Mycorrhizae

Mycorrhizae are fungi that form a symbiotic association with plant roots. In endomycorrhizal relationships the fungi penetrate and exchange nutrients with root epidermal cells. *Ectomycorrhizal* fungi grow across the root surface and between root cortical cells. Mycorrhizae, especially *endomycorrhizae*, benefit plants by increasing the de facto surface area of roots for water and nutrient absorption (Biermann and Linderman 1981). Mosse, Hayman, and Arnold (1973) showed mycorrhizal infection was especially important for phosphorus (P) uptake by *Paspalum notatum* in P-deficient soils. Mycorrhizae also may provide other benefits to infected plants. Dueck et al. (1986) reported that vesicular-arbuscular (VA) mycorrhizae (*Glomus fasiculatum* Gerdemann and Trappe) decreased zinc toxicity in red fescue (*Festuca rubra*) and bush grass (*Calamagrostis epigejos*), resulting in increased biomass of roots compared with noninfected plants. Although enhanced nutrient uptake typically is attributed to mycorrhizal infection, the mycorrhizae decreased translocation of iron, manganese, and potassium from the roots to the shoots in certain instances and enhanced it in others. Zinc uptake and translocation was not affected significantly by mycorrhizae in either grass species.

Mycorrhizal associations and effects are likely to vary among grass and fungal species. Although few data are available for mycorrhizal effects on grasses compared with woody plants, data indicate mycorrhizae are important for warm-season grasses but have neutral or negative effects on cool-season grasses (Brejda et al. 1993; Hetrick, Wilson, and Todd 1990; Wilson and Hartnett 1997). Specific unknown variables include the extent and types of mycorrhizal infection in grasses, factors involved in the infection process, and the proportional benefits accrued by grasses. Gay, Grubb, and Hudson (1982) reported seedlings of *Festuca ovina* and several nongrass species became naturally infected in a field experiment within 2 weeks of germination. The amount of mycorrhizal infection in the grass species studied varied by season and year. Because nonmycorrhizal controls were not included, the importance of the mycorrhizae could not be determined. In well-fertilized and irrigated swards mycorrhizae may play an insignificant role in grass growth and development, particularly if systemic fungicides are applied routinely, which may impair mycorrhizal growth.

Several methods have been used by researchers to quantify vesicular-arbuscular (VA) mycorrhizae in plant roots. Becker and Gerdemann (1977) used colorimetric procedures (*colorimetry*) to measure pigmentation associated with mycorrhizae, and Hepper (1976) used these procedures to measure the conversion of fungal chitin to glucosamine. Most researchers have relied on microscopic examination of tissues. Methods have ranged from recording the presence or absence of fungi in root segments (Nicholson 1955; Read, Koucheki, and Hodgson 1976), to grid measurements (Ambler and Young 1977; Davis, Menge, and Erwin 1979,) to entire root systems (Ames and Linderman 1977). Based on their own investigations of mycorrhizae in three plant species, including the perennial timothygrass (*Phleum pratense* L.), Biermann and Linderman (1981) proposed a standard quantification method to overcome limitations of previous methods. They used a clearing-staining procedure to develop frequency distributions of fungal infections in roots. Their data indicated a minimum of seven samples composed of 25 root segments (0.5 to 1.0 cm) provided confidence limits within 10% of the mean. The primary shortcoming of most methods is their inability to correlate the presence of mycorrhizae with activity.

Rhizobia

Rhizobia are nitrogen (N)-fixing bacteria (*Rhizobium* spp.) surviving in root nodules of leguminous plants in a symbiotic relationship. Although *Rhizobium* do not form nodules with grasses, certain free-living bacteria such as *Azospirillum* spp. are capable of fixing N for plant uptake and may exist in the rhizosphere of many plant types, including grasses. As with other microbes, the mechanisms for coexistence and attractions with grass hosts have not been studied well. Little if any published research is available on the impacts of BD grasses on rhizobia or other microbes. In Australia, the occurrence of herbicide resistance in grasses and increased disease pressures in legumes may force the adoption of specific rotational schemes, which may impact the presence of rhizobia (Howieson 1995).

6 Questions and Answers: A Summary of Workshop Responses and Public Comments

The workshop "Biotechnology-derived, Perennial Turf and Forage Grasses: Criteria for Evaluation" was designed to provide a forum to discuss the State-of-the-Science of biotechnology-derived (BD), perennial turf and forage grasses, as well as to begin a dialogue on possible criteria used to determine the environmental safety and potential benefits and risks of these grasses relative to traditional varieties. Multiple opportunities to provide comments were provided throughout the workshop, including two public comment sessions and three breakout sessions to facilitate discussion of key questions regarding the evaluation of BD perennial turf and forage grasses. Additional public comments were solicited actively by the Council for Agricultural Science and Technology (CAST), and submissions were accepted through February 15, 2003, after completion of the conference.

There was a wide diversity of opinion regarding how BD perennial grasses should be developed. Many concerns were interesting but not based on scientific facts, or simply could not be tested in a reasonable way. It was obvious from the comments that certain groups were animatedly against the release of all BD crops, including perennial grasses, whereas others believed the deregulation process was overly burdensome and limited innovation. It would be difficult to recommend evaluation criteria to satisfy such a diverse group; however, the goal of this chapter is to at the very least give the U.S. Department of Agriculture (USDA), the U.S. Environmental Protection Agency (EPA), and the U.S. Federal Drug Administration (FDA) a starting point on which existing environmental risk assessment criteria can be adapted for BD perennial grasses.

The criteria for evaluation of BD plants intended for unconfined release fall into three major categories: flowering, seed, and vegetative characteristics. This chapter was assembled from the workshop discussions and public comments provided to the Task Force. The initial draft of this section was prepared by the cochairs and subsequently reviewed on two occasions by the 11-member Task Force who authored the other chapters of this publication. The panel of reviewers selected by CAST also evaluated the answers to these questions.

This chapter primarily answers the questions posed by the CAST Steering Committee responsible for the overall planning and implementation of the Workshop. The answers include information on the reason behind why a specific number of years, locations, or sampling periods were recommended. Often an answer provides a current standard based on current research on perennial grasses in North America. Unfortunately, there is some redundancy in the questions and answers throughout this chapter, and in response to comments from several reviewers, the flowering properties and seed production sections were combined inasmuch as these biological processes are related closely.

Background Information

Petitioners seeking to have a BD or genetically engineered organism deregulated under Animal and Plant Health Inspection Service (APHIS) regulations (7CFR 340.6) are required to describe the known and potential differences from the unmodified parental organism that would substantiate that the regulated organism is not likely to pose a greater plant pest risk than the unmodified organism from which it was derived.

Over the past decade, the APHIS has provided additional information on data requirements in addition to that given in the regulations. One goal of this workshop was to get input on what types of data would be necessary to address questions concerning perennials, such as turf and forage grasses. The workshop focused primarily on the phenotype of the transgenic plant. This discussion included a broad list of observable characteristics that would indicate any biologically significant change in plant morphology and reproductive and survival biology. Unintended effects of *somaclonal variation* and the engineering procedures also could be detected. Although there is considerable scientific debate on whether these specific characteristics can be used as predictors of invasiveness or weediness, the lack of significant change between the engineered plant and its parent would provide strong support that there has been no change in these important biological parameters.

Issues Related to the Flowering Properties and Seed Production Characteristics of Biotechnology-derived, Perennial Turf and Forage Grasses

Flowering Date, Flowering Period, Days to Maturity, and Seed Production

Flowering properties and seed production are important to consider because of the impact new varieties could have on existing varieties and native relatives in the vicinity of commercial production fields. The first set of questions deals with determining if the BD perennial grass has flowering properties or seed production characteristics that differ from existing varieties for the same species. Flowering characteristics include the date of the onset of flowering, the number of days for the flowering period, and the number of days to maturity. Seed production deals primarily with the amount of seed produced by individual plants or for a given area.

What number of testing sites and locations should be recommended for the critical evaluation of flowering properties and seed production in BD perennial grasses?

Flowering properties and seed production can be assessed by collecting data for a minimum of 2 years (growing seasons) at multiple locations (two or more) within the normal range of adaption. This minimum guideline of four environments (location-years) is a more cautious approach than accepted scientific experimental procedures for peer-reviewed publications and federal plant variety protection (PVP), data collection for 2 years at one location or two locations for 1 year (two environments or location-years). Increasing the minimum recommendation for the evaluation of BD perennial grasses to data collection during two growing seasons would allow researchers time to examine plants over two full flowering and seed production cycles. Testing in a minimum of two locations would allow scientists to observe plants in environments with a different climate and soil.

Should there be a minimum number of test sites <u>within</u> the normal geographic distribution range of the grass species under consideration? If so, what number of test sites would be an adequate minimum?

There is a diversity of opinion that ranges from a few locations to every location where the BD perennial grass will be produced. The number of test sites *within* the normal geographic range should be determined on a case-by-case basis, however, considering the species, gene of interest, intended use, and existing body of knowledge regarding these three considerations. For example, if the inserted gene does not increase the known adaptation of the perennial grass, and there is no indication that flowering properties or seed yield are elevated in a single test location optimized for these characteristics, then the potential for unusual flowering or increased seed production as a result of the inserted trait(s) is of little concern. But the number of locations and environments will need to be increased to address the potential for expanding the range of seed production of the BD grass in situations where the inserted gene is known to increase its adaptation dramatically.

Should there be a minimum number of test sites <u>outside</u> the normal geographic distribution range of the grass species under consideration?

No additional evaluations for flowering properties or seed production should be required outside the normal geographic range of the species unless the introduced trait is expected to change the environmental adaptation of the BD perennial grass. In cases where the introduced trait will increase the environmental adaptation, additional evaluations outside the normal geographic range are warranted. But the number and location of sites outside the normal range of adaptation will need to be determined on a case-by-case basis to address the improved fitness potential of the introduced trait.

What is the appropriate planting system to use to evaluate seed yield of these plants properly?

Cool-season perennial grasses typically are evaluated as spaced plants for PVP submission standards rather than solid or *drill rows*. Spaced plants mimic a more naturalized setting and are more conducive to identifying differences between *cultivars*. For warm-season grasses, space-plant or defined areas of established grass (field plots) can be used. When defined areas are used, data may be collected on several 1-square-foot sampling areas.

What should be the recommended duration of testing periods for critical evaluation of flowering properties or seed production in BD perennial grasses?

Tests should be run over the course of a normal

flowering cycle for the species of interest. The flowering cycle for the species of interest is determined by its response to *vernalization*, *induction*, or *initiation stimuli*. Perennial grasses achieve maturity when they are receptive to vernalization or floral induction. Floral induction of cool-season grasses (vernalization) occurs in autumn as days become shorter and temperatures drop. During induction, physiological changes occur that promote flowering to take place in the grass plant in response to colder temperatures. Floral initiation and development occur in spring as days become longer and temperatures rise. Warm-season grasses have an *indeterminate flowering* period that occurs during the late spring through early fall and do not require cold temperature induction in order to flower.

For cool-season species, tests can be run with newly initiated, spaced plants for 1 year or during the next growing season for fall plantings provided they are mature. Warm-season species should be tested from late spring through early fall a year after establishment. Warm-season species need to form a solid sod before they will produce a large number of flowers.

Should data be collected on perennial grasses annually or more frequently for seed production?

The frequency of data collection is dependent on the species and what already is known about its flowering habit and seed productivity. For those grasses that have determinate seed set, data should be collected annually during flowering and seed production for a minimum of 2 years at two locations. For indeterminate seed set, data should be collected two to three times during the growing season for 2 years at two locations. These proposed testing periods take a more cautious approach, which exceeds the current PVP standard of two location-years.

How old should the plants be when data are collected for critical evaluation of flowering properties and seed production in biotechnology-derived, perennial grasses?

The age of the plants for flowering properties and seed production evaluation is dependent on the flowering life cycle. For cool-season species, the test should be conducted using newly initiated spaced plants that are mature and receptive to vernalization. Because *tillers* only survive for one season and die after flowering, the second year data can be collected from new tillers produced during the previous growing season. A typical seed production system is planted in the fall and harvested the following spring. Warm-season perennial grasses should be tested from late spring through early fall a year after establishment. Warm-season grasses have an *indeterminant flowering* habit that will require multiple evaluations during the year to establish seed production characteristics.

Indeterminate Flowering

Indeterminate flowering deals with the length of time in days that flowering and seed production occur. Observations of BD perennial grasses need to document that there are no significant changes from existing varieties.

For those grasses that do not have one well-defined, annual seed production period, what additional data considerations need to be addressed?

Perennial grasses with indeterminate flowering should be harvested two to three times during the growing season. Seasonal flower initiation (heading date) and *anthesis* (pollen release) dates should be recorded for grass species that do not have well-defined or determinate annual seed production periods. By requiring multiple observations during the growing season, the period and quantity of flowers during a growing season can be compared with existing cultivars of the same species.

Outcrossing Frequency

To determine the outcrossing frequency within the same species as well as the outcrossing frequency among related species normally found in the United States requires that several questions be answered.

How many testing sites and locations should be recommended for critical evaluation of outcrossing in biotechnology-derived, perennial turf and forage grasses?

The scientific literature should be consulted to determine if outcrossing studies are necessary. If this literature is lacking appropriate documentation, then the frequency of hybridization should be determined under controlled experimental conditions. If controlled studies suggest that hybrid formation is possible, that hybrids are problematic weeds, and that environmental adaptation and weediness of the primary transformed species has been impacted, then environmental risks of the putative hybrids should be assessed. Such assessments should use the scientific literature available for the species, the likelihood of hybridization, management programs to limit hy-

brids, mitigation difficulty, and the expected selective advantage, if any, conferred by the trait in question. It *is not* recommended to conduct a wide range of field studies to determine hybrid formation in natural settings. The information required to estimate the degree of natural hybridization can be obtained with better accuracy in controlled studies at field experiment stations. In addition, molecular techniques can be used on field collections to look for hybridization (Saltonstall 2002).

Should there be a minimum number of test sites within the normal geographic distribution range of the grass species under consideration? If so, how many test sites would be an adequate minimum?

At least 2 years at three locations should be tested if scientific studies suggest that hybrids will occur, that the hybrids will be recognized widely as an economically problematic weed, and that the environmental adaptation or weediness of the BD perennial grass has been increased.

Should there be a minimum number of test sites outside the normal geographic distribution range of the grass species under consideration?

One location should be tested if scientific studies indicate that hybrids will occur, that the hybrids will be problematic weeds, and that the environmental adaptation or weediness of the BD perennial grass has been increased.

Should data be obtained from well-established, unmowed, spaced plants or from solid-seeded rows with no competition from any surrounding plants?

For cool-season perennial grasses, spaced-plant evaluation of plants rather than *drill* (seeded) *row* are required for PVP submission standards. Spaced plants mimic a more naturalized setting and are more conducive to identifying differences between cultivars. For warm-season species, spaced-plant or defined areas of established grass (field plots) can be used. When using defined areas, data should be collected on several 1-square-foot sampling areas.

What should be the recommended duration of testing periods for the critical evaluation of outcrossing in biotechnology-derived, perennial turf and forage grasses?

Perennial grasses achieve maturity when they are receptive to vernalization or floral induction. Tests should be run over the course of a normal flowering cycle for the species of interest. The flowering cycle for the species of interest is determined by its response to vernalization, induction, or initiation stimuli. For cool-season species, the tests can be run with newly initiated, spaced plants for 1 year or during the next growing season for fall plantings provided they are mature. Warm-season species should be tested from late spring through early fall 1 year after establishment.

Should data be collected annually or more frequently?

The frequency of data collection is dependent on the species and knowledge of flowering habit. For determinate flowering, 2 years at two locations is recommended and exceeds the current PVP standard of two location-years. For indeterminate flowering, data should be collected two to three times during the growing season for 2 years at two locations.

How old should the plants be when data are collected for critical evaluation of outcrossing in biotechnology-derived, perennial turf and forage grasses?

The age of the plants for outcrossing experiments is dependent on the flowering life cycle. For cool-season species, the test should be conducted on newly initiated spaced plants that are mature and receptive to vernalization. Because tillers only survive for one season and die after flowering, the second year data can be collected from new tillers produced during the previous growing season. A typical seed production system is planted in the fall and harvested the following spring. Warm-season perennial grasses should be tested from late spring through early fall beginning after the year of establishment.

Should these tests be conducted under natural conditions or in a contained facility, such as a greenhouse or growth chamber?

The tests can be conducted in natural conditions, greenhouses, or growth chambers as long as the conditions optimize the environment for flowering to occur.

If interspecific and/or intergeneric hybrid seeds are formed, should additional studies be conducted—such as seed germination and growth of hybrid plants—to determine vegetative vigor and seed fertility?

First, it is assumed that if there is no scientific evidence that the transgene will impact the growth and development of the primary transformed species, then there is no expectation that hybrids containing

the transgene will be any different from hybrids formed with conventional varieties. If there is a scientific basis to expect that hybrids will be better adapted, then suspected (putative) hybrid seed should be germinated to observe the frequency with which hybrids occur. During this testing, information on seed germination and seedling vigor could be obtained. If hybrids do occur, data should be collected on the vegetative vigor and fertility of these plants.

How many generations should be followed after the initial hybrid seed is formed?

One generation should be evaluated if there is scientific evidence that indicates hybrids will occur, that the hybrids will be problematic weeds, or that the environmental adaptation or weediness of the BD perennial grass has been increased.

What type of studies should be conducted with these hybrid plants and succeeding generations?

The evaluations discussed previously on one generation will be sufficient to determine if hybrids will become problematic weeds.

Impact on Pollinator Species and Associated Species

What type of studies, if any, should be conducted to determine the impact on pollinator species and other associated species?

Most perennial grasses are wind pollinated, so studies evaluating insect pollination would not be necessary for these species. In instances where BD perennial grasses are known hosts for insect pollinators, then studies suggested by the APHIS for other BD crops would be appropriate.

Pollen Parameters

What type of studies should be conducted to evaluate pollen parameters such as viable pollen production, amount of pollen produced, proportion of viable pollen, duration of pollen viability, and physical parameters (e.g., size, shape, stickiness, weight)?

Pollen size and viability should be examined to determine morphological and adaptation changes. Bagged crosses from *hemizygous* plants and non-transgenic plants could be grown out to determine *segregation ratios* and ability to effect pollination. If segregation is 50:50 with plants that breed normally, then pollen viability is not impacted by the transgene.

Which of these parameters might affect the viability or performance of the pollen?

The size, viability, or longevity of pollen may impact fertility. These factors could be evaluated along with examining the ability to outcross in controlled crossing experiments.

Fertility

What type of studies should be conducted to determine whether plants have acquired or lost fertility?

Fertility is an indication of the ability of a plant or variety to produce offspring. Controlled crosses and grow-outs can be used to determine fertility. Evaluating seed productivity is a good indicator of female fertility, whereas evaluating pollen viability indicates male fertility.

Self-Compatibility

What type of studies should be conducted to determine if self-compatibility changed from the standard?

Self-pollination studies should be conducted by growing the plants in isolation or bagging individual *inflorescences*.

Asexual Reproduction

What type of studies should be conducted to determine if asexual reproduction has changed from the standard?

Apomictic species produce seed asexually, a situation in which progeny of a BD perennial grass could be grown out under controlled conditions to compare with an *isoline*. Seedlings of both parent plants should be examined to determine the number of plants that are true to type. The variability among the progeny compared with the expected variability would provide an indication or whether or not asexual reproduction has changed.

Male Sterility

Will male sterility increase, decrease, completely eliminate, or have no effect on gene flow from the transgenic plant to neighboring related species?

Male sterility has been suggested as a means to eliminate gene flow from the transgenic plant to related wild species. Male sterility would help prevent

BD perennial grasses from outcrossing with wild relatives or related species. Male-sterile plants could be produced vegetatively and then allowed to open-pollinate with the preferred variety. The advantage of this system is that transgene escape could be reduced. But gene flow still will occur between related species because reciprocal crosses are possible. Male sterility will prevent the flow of pollen from the BD perennial grass to other fields or native grasses; however, pollen still will flow into the BD perennial grass from these outside sources. Additional studies are needed to determine the efficacy of the current male-sterile systems.

Seed Dormancy

Seed dormancy deals with the ability of the seed to remain viable over time without germinating even when subjected to conditions favorable for germination.

What tests should be recommended to address seed dormancy issues?

Seed dormancy is an important issue because of (1) poor or unsatisfactory establishment and (2) the potential movement from the intended planting site. Standard seed testing procedures, as outlined by the Association of Official Seed Analysts (AOSA), are recommended. For example, the tetrazolium (TZ) and AOSA standard germination tests will provide information to address seed dormancy issues and have been used for many years to examine these issues. For species with low germination rates, removing the *florets* (hulled) or *scarification* may be necessary to discern seed germination.

Seedling Emergence

Seedling emergence measures the proportion of seeds planted that emerge as seedlings; however, there often are several stresses that will decrease drastically the number of seedlings that emerge. Seedling survival to reproduction is a better measure of the overall fitness of a variety to pass along genetic information to the next generation because this process includes the production of fertile flowers and viable seed. Survival to reproduction will involve the continuation of tests conducted for seedling emergence, however, which, depending on the species, may be compromised because of the potential for a significant amount of seedling mortality that can impact variability significantly. Therefore, studies should be designed on a case-by-case basis to ensure reliable measurements of seedling emergence and/or reproduction.

What tests should be recommended to address seedling emergence issues?

Most cool-season turfgrasses have high germination and seeding emergence (greater than 80% germination), whereas warm-season species tend to have lower germination and emergence. It is recommended that an AOSA standard germination test for the species be conducted. If no significant differences are detected, then it is unlikely that germination has been impacted by the gene in question or transformation process. If significant differences are observed, however, then an evaluation of the impact of increased or decreased seedling emergence will need to be addressed. Also, a field test under representative environments for which the species will be used should be conducted to determine seedling emergence and field survival. Field tests should be conducted on both bare soil and in existing turf or forage grasses. Bare soil field survival tests will provide information in a noncompetitive environment, whereas tests conducted in established grasses indicate the ability of seedlings to compete with mature grass plants.

Should these tests be conducted in a minimum number of different environments?

For laboratory seed germination tests, the AOSA recommends a temperature range of 14° C to 32° C as a low and high end for optimal germination. For field tests, a minimum of 2 years and two environments for areas where the species is adapted will be sufficient because this will provide enough environmental variation to determine field survival.

Should this testing include only studies in managed turf or forage situations, or should studies be included outside of these situations managed for turf or forage?

For field survival, tests should be conducted under representative environments that include both noncompetitive bare soil and competitive turf or forage situations. Bare soil and the turf or forage tests include the competitive extremes that BD perennial grass seed will experience.

Proportion Surviving from Seedling Emergence to Reproduction

Grass seedlings face many obstacles en route to becoming mature plants capable of producing seed. The actual proportion of seed that germinate and survive to produce seed is quite low. A series of tests, however, are needed to document that the BD perennial grass has not changed significantly from stan-

dard varieties for its ability to survive from seeding emergence to reproduction.

What would be the recommended number of testing sites, locations, and testing periods to determine the proportion of seedlings surviving to reproduction?

An experiment addressing this question would be difficult to conduct because of a range of uncontrolled variables (e.g., temperature, soil, precipitation, water quality, etc.). The information could be obtained, however, through a series of experiments that examines the perennial grass life cycle (e.g., germination/emergence, vegetative growth, flowering, seed production) in two to four environments (location-years). Additional sites may be necessary for transgenes that are expected to increase the range of adaptation of the perennial grass species.

Standards for Comparison

Standards for comparison are needed to evaluate if the BD perennial grass has changed significantly from existing varieties. The standards for comparison are needed to satisfy Regulation 7CFR340.6 (c) (4) (USDA–APHIS 1997), which requires the applicant to describe known and potential differences of the regulated article from the unmodified recipient organism.

Should BD turf and forage grasses be evaluated in comparison with isolines or other appropriate varieties?

Isolines do not exist for most perennial grass species. For grasses that produce asexually through *apomixis*, however, there would be an opportunity to provide plants that would approach an isoline. Regardless, BD perennial grasses should be compared with a set of plants representative of the range of performance in the agronomic and phenotypic characteristics of a species that may include conventional cultivars and *null segregants*. Null segregants are progeny from parent plants closely related to the transformed parents used to develop the BD perennial grass variety.

Where an isoline or comparable unmodified recipient organism is not available, what is the appropriate comparator?

Null segregants from sexual crosses in populations segregating for the trait are recommended. Taxonomic descriptions from manuals would provide supplemental comparisons to determine whether the BD perennial grass falls outside the known description of the species.

Breeding Behavior and Seed Development

The perennial grasses used for turf and forage purposes may range from self-pollinated to *cross-pollinated*; from self-compatible to self-incompatible; from sexual to obligate apomictic seed production; from belonging to a genus with no other closely related species, to a genus with only one or two closely related species, to a genus with many closely related species; and from normally seed-propagated to sterile-seeded, vegetatively propagated.

How would these differences affect the recommended data requirements?

Except for plants that are confirmed to be both male- and female-sterile, the data requirements should be the same for all seed production methods. Seed and flowering studies would not be required for sterile plants.

Issues Related to Vegetative Properties of Biotechnology-derived, Perennial Turf and Forage Grasses

Growth Habit

Growth habit should be observed for changes in basic morphology and plant architecture, including any abnormalities. This will be important especially for characteristics dealing with the spread of BD perennial grasses by vegetative organs such as *stolons* and *rhizomes*. Also, the ability to survive extremes in temperature and drought are important to determine if the BD perennial grass will survive outside its normal range of adaptation.

Should the data be obtained from well-established, unmowed, spaced plants growing in bare soil; and/or in a normal managed turf or forage situation in competition with other normal grass species with which they normally are associated and/or in a setting other than for managed turf or forage such as an untilled, unmowed area; and/or in another setting?

Biotechnology-derived, perennial grasses should be evaluated in a range of environments that repre-

sent seed production and their intended use. Comparisons should range from bare soil to existing perennial grass *swards* (continuous areas of grass.) These conditions will enable an accurate assessment and comparison of plant morphology and vegetative growth.

Because of environmental variability, what period of time should be used to obtain growth habit data?

Two growing seasons and two locations in both bare soil and competitive environments should provide an indication of general increases in vegetative growth capacity.

How many locations within and outside the normal geographic growing range of the grass should be used in order to evaluate the impact of climate on growth habit characteristics accurately?

Three locations within the geographic range are recommended to evaluate the potential impact of climate on growth habit characteristics. One location outside the range of the traditional growing range is suggested to document that the grass has not obtained the ability to survive outside its normal growing range.

Which plant characteristics should be measured, and how often should the data be collected?

Plant spread and percentage of groundcover should be evaluated for nondestructive, long-term studies, whereas total shoot biomass should be obtained for endpoint evaluations. Nondestructive estimates will provide information on the rate of growth for individual plants over the active growing season. Shoot biomass provides an endpoint estimate of how efficiently the plant converted the available water, nutrients, and light into plant tissues during the growing season.

Vegetative Vigor

Two characteristics often associated with increased potential for weediness are rapid growth and vigorous vegetative growth. Perennial grasses, in general, probably are well adapted in natural settings because of their vegetative growth characteristics.

Should the data be obtained from well-established, unmowed, spaced plants growing in bare soil; and/or in a normal managed turf or forage situation in competition with other normal grass species with which they normally are associated; and/or in a setting other than for managed turf or forage such as an untilled, unmowed area; and/or in another setting?

Biotechnology-derived, perennial grasses should be evaluated as spaced plants in bare soil to obtain the most accurate estimate of vegetative vigor and as spaced plants in competitive vegetative situations to provide an indication of plant competitiveness.

In the special instance of herbicide-tolerant grasses, should the data be obtained from a cropping system that has the same introduced trait in order to test whether the BD grass would express its tendency for "weediness?" For example, if ryegrass is engineered to be resistant to Herbicide A, should it be tested in a crop such as soybeans that has been engineered to be resistant to Herbicide A?

The concept of familiarity should dictate the necessity of this type of research trial. If the BD perennial grass species is not recognized widely as an economically problematic weed, then these tests are not needed. If the adaptation of the BD perennial grass has been changed significantly, however, and other seed production and vegetative tests document this change, then these studies should take place.

Because of environmental variability, what period of time should be used to obtain vegetative vigor data?

A minimum of two growing seasons at three locations within the range of adaptation and two growing seasons at one location outside the range of adaptation will provide enough environmental variation to determine vegetative vigor. Data collected during two growing seasons at multiple locations (three or more) provide sound scientific basis for assessing the agronomic characteristics. This recommendation of six environments (location-years) would entail an even more cautious approach than accepted scientific experimental procedures for peer-reviewed publications and federal PVP requirements, which is data collection for only 2 years at one location or two locations in 1 year (two environments or location-years). The recommendation that data should be collected during two growing seasons would allow researchers to examine plants over two full growing seasons. Testing in three locations would allow scientists to observe plants in environments with a different climate and soil. An additional 2-year test outside the normal range of adaptation will substantiate that the

BD perennial grass has not been altered in a way that allows it to survive.

How many locations within and outside the normal geographic growing range of the grass should be used in order to evaluate the impact of climate on vegetative vigor characteristics adequately?

Three locations within the normal geographic range are recommended to evaluate the potential impact of climate on vegetative growth characteristics. One location outside the range of the traditional growing range is suggested to document that the BD perennial grass has not been altered in a way that allows it to survive outside its normal growing range.

Which plant characteristics, such as plant width, height, and/or fresh weight, should be measured, and what should be the data collection frequency for each plant characteristic measured?

Plant spread and percentage of groundcover should be obtained for nondestructive, long-term tests, whereas total shoot biomass should be recorded at the end of the experiment. Nondestructive estimates will provide information on the rate of growth for individual plants over the growing season. Shoot biomass provides an endpoint estimate of how efficiently the plant converted the available water, nutrients, and light into plant tissues during the growing season.

Life Span

Has the introduced gene(s) produced changes that affect the fitness of the plant such that the plant has a longer or shorter useful life?

By their nature, perennial grasses have long life spans, and whereas it would not be necessary to evaluate the life span, evaluating a BD perennial grass over two locations and multiple environments would provide the necessary information on the ability of the grass to persist for long periods.

What is the recommended procedure for obtaining data on life span?

Characteristics that document the different life cycle phases of the perennial grass species need to be measured. If these characteristics are collected over time in different environments, the information will provide an adequate estimate of the ability of the BD perennial grass to persist for extended periods.

Ability to Overwinter or Overseason

A plant's ability to tolerate different extremes in heat and cold is a major factor in determining its growth range, with grasses broadly categorized into warm- and cool-season grasses. Determining cold and heat tolerance generally is difficult. Both field and laboratory evaluations are possibilities.

For field evaluations, what would be the recommended number of testing sites, their locations, and testing periods?

A tiered testing approach is recommended. All BD perennial grasses should be tested in a minimum of three locations within the normal range of adaptation and one location outside of the range of adaptation (i.e., first tier). If there is evidence that the introduced gene increases the adaptation of the BD perennial grass species, then the number of field evaluations would need to be reexamined. There may be cause to expand the number and range of tests to document how the BD perennial grass has changed its ability to overwinter or overseason (i.e., second tier). If the adaptation of the BD perennial grass is not significantly different from conventional cultivars, however, then it is not necessary to get survival endpoints from more than one environment outside the normal range of adaptation.

Should laboratory procedures be acceptable? If so, what should be the standard for acceptability?

The scientific literature documents a range of methods to estimate field adaptation developed using growth chambers and greenhouses. Whether the tests deal with abiotic stresses because of pathogens and insects, or with biotic stresses involving cold, heat, drought, or salinity, plant scientists have developed testing procedures that provide excellent estimates of survival rates. The point of these tests is to determine whether the BD perennial grass differs significantly from conventional cultivars. These tests should follow the experimental design, materials and methods, and protocols outlined in the scientific literature.

Symbionts

Symbionts include organisms such as *mycorrhizae*, *rhizobia*, *endophytes* known to live in close association with perennial grasses. Observations are needed to make sure that symbiotic organisms have not been altered.

What type of studies should be conducted to determine if there have been any effects on symbionts in grasses? If effects are noted, how are the impacts on growth and reproduction of the grass and the effects on animals that consume this grass determined?

Information regarding symbionts in perennial grasses should be required if there is evidence that such organisms have been altered genetically by the transformation method. In addition, alterations in the known relationships between the BD perennial grass and symbionts need to be documented.

Stress Adaptations

Biotic stress factors include parasites, pathogens, competitors (weeds), herbivores, etc. Abiotic stress factors include moisture, heat, cold, nutrient deficiency, etc.

What type of studies should be conducted to determine if there have been any effects on stress adaptations in BD grasses to date? For how long?

Abiotic and *biotic stress* resistance information is evaluated during the routine monitoring of all field tests of BD plants as required under the conditions of the USDA notification or permit. If there is evidence that the introduced gene increases the adaptation of the BD perennial grass species, then the number of field evaluations would need to be reexamined. There may be cause to expand the number and range of tests to document how the BD perennial grass has changed its ability of overwinter or overseason. If adaptation of the BD perennial grass is not different significantly from conventional cultivars, however, then it is not necessary to get survival endpoints from more than one environment outside the normal range of adaptation.

As pointed out earlier, a range of methods to estimate field adaptation has been developed using growth chambers and greenhouses. These tests are documented in the scientific literature and reveal differences in plant material for several abiotic and biotic stresses. Growth chamber and greenhouse testing is a good estimate to determine if further field testing is necessary. The tests should be conducted to determine if the BD perennial grass differs significantly from conventional cultivars.

Standards for Comparison

Standards for comparison are needed to evaluate if the BD perennial grass has changed significantly from existing varieties. The standards for comparison are needed to satisfy Regulation 7CFR340.6 (c) (4) (USDA–APHIS 1997), which requires the applicant to describe known and potential differences of the regulated article from the unmodified recipient organism.

Should biotechnology-derived, perennial turf and forage grasses be evaluated in comparison with isolines or other appropriate varieties?

Isolines do not exist for most perennial grass species. But for grasses that produce asexually through apomixis, there would be an opportunity to provide plants that would approach being isolines. Regardless, BD perennial grasses should be compared with a set of plants representative of the range of performance of the agronomic and phenotypic characteristics of a species, which may include conventional cultivars and null segregants. Null segregants are progeny from parent plants closely related to the transformed parents used to develop the BD perennial grass variety.

Where an isoline or comparable unmodified recipient organism is not available, what is the appropriate comparator?

Because isolines are not appropriate, BD perennial grasses should be compared with a set of plants representative of the range of performance in the agronomic and phenotypic characteristics of the species, which may include conventional cultivars and null segregants.

Appendix A: Web Resources

Plant Variety Protection Act Documents

A.1. USDA/Agricultural Marketing Service. Plant Variety Protection Act and Regulations and Rules of Practice. USDA, revised March 2001. 53 pp. <http://www.cast-science.org/turfappendixA/Appendix A.1 Plant Variety Protection Act and Reulations and.pdf>

A.2. USDA/Agricultural Marketing Service/Science and Technology—Plant Variety Protection Office. Application for Plant Variety Protection Certificate/Form ST-470 (04-03). 2 pp. <http://www.cast-science.org/turfappendixA/Appendix A.2 Application for Plant Variety Protection Certif.pdf>

A.3. USDA/Agricultural Marketing Service. Objective Description of Cultivars, Bermuda grass. 9 pp. <http://www.cast-science.org/turfappendixA/Appendix A.3 Objective Description of Cultivars - Bermudagra.pdf>

A.4. USDA/Agricultural Marketing Service. Objective Description of Variety, Tall and Meadow Fescues (Festuca spp.). 5 pp. <http://www.cast-science.org/turfappendixA/Appendix A.4 Objective Description of Variety, Tall and Mead.pdf>

A.5. USDA/Agricultural Marketing Service. Exhibit E: Statement of the Basis of Ownership. Form ST-470-E (04-03). 1 p. <http://www.cast-science.org/turfappendixA/Appendix A.5 Statement of the Basis of Ownership.pdf>

Appendix B: Link to Public Comments

The workshop "Biotechnology-derived, Perennial Turf and Forage Grasses: Criteria for Evaluation" was held in Baltimore, Maryland, on January 9–10, 2003. Multiple opportunities for public comment were provided before, during, and for more than one month after the workshop. Beginning with the first public announcement of the workshop, the organizers identified opportunities for public input with the following statement:

> In addition to providing input during two public comment periods, Q & A sessions, and three breakout sessions during the workshop, members of the public may submit data, background information, and other comments through February 15, 2003. Public input is encouraged. Send input to <crichard@cast-science.org> or C. Richard, CAST, 505 Capitol Court, N.E., Suite 200, Washington, DC 20002.

Links to the public comments received in conjunction with the workshop may be found at <http://www.biotech-cast-science.org/Meetings.htm>. Links are organized in the order the comments were received.

Comments received prior to the January 9 and 10, 2003 workshop

Comments received during public comment sessions at the workshop

Summaries of facilitated discussions during concurrent breakout sessions

Comments received or postmarked between January 11 and February 25, 2003

Appendix C: Workshop Participant List

Jim Aidala
Stone & Webster–JSC Management Consultants, Inc.
TEL: 202-261-1900
FAX: 202-261-1950
Jim.Aidala@shawgrp.com

Carol Auer
University of Connecticut
TEL: 202-720-2662
FAX: 202-720-1815
cauer@oce.usda.gov

Laura Bartley
USDA–BRS
TEL: 301-734-5720
FAX: 301-734-8669
laura.bartley@aphis.usda.gov

Stefi Baum
Dept. of State, Economics and Business Bureau
TEL: 202-647-4232
FAX: 202-647-1894
baumsa@state.gov

Rebecca Bech
USDA–APHIS
TEL: 301-734-7324
FAX: 301-734-5928
Rebecca.A.Bech@usda.gov

Prasanta C. Bhowmik
University of Massachusetts–Amherst
TEL: 413-545-5223
FAX: 413-545-3958
pbhowmik@pssci.umass.edu

Michael Boslet
Golfweek's Superintendent News
TEL: 407-563-7040
FAX: 407-563-7077
mboslet@golfweek.com

John Bosser
The Scotts Company
TEL: 937-578-5615
FAX: 937-578-5751
john.bosser@scotts.com

Leah A. Brilman
Seed Research of Oregon
TEL: 541-758-9115
FAX: 541-752-2065
srofarm@attglobal.net

Arthur Bruneau
North Carolina State University
TEL: 919-515-5854
FAX: 919-515-5855
Art_Bruneau@ncsu.edu

Suleiman Bughrara
Michigan State University
TEL: 517-432-8017
FAX: 517-353-5174
bughrara@msu.edu

Michael D. Casler
USDA–ARS–U.S. Dairy Forage Research Center
TEL: 608-264-5279
FAX: 608-264-5147
mdcasler@wisc.edu

Douglas J. Cattani
Pesticide Management Regulatory Agency
TEL: 613-736-3812
FAX: 613-736-3770
Doug_Cattani@hc-sc.gc.ca

Linda Chimenti
CAST
TEL: 515-292-2125, ext 31
FAX: 515-292-4512
lchimenti@cast-science.org

John M. Cordts
USDA–APHIS
TEL: 301-734-5531
FAX: 301-734-8669
John.M.Cordts@aphis.usda.gov

David Crow
D.C. Legislative and Regulatory Services
TEL: 202-454-5282
FAX: 202-454-5252
dcrow@dclrs.com

Joseph Duich
Pennsylvania State University
TEL: 814-863-3460
FAX: 814-237-5226

Terri G. Dunahay
USDA–APHIS–BRS
TEL: 301-734-4308
FAX: 301-734-8669
terri.g.dunahay@usda.gov

Timothy E. Dutt
Monsanto Company
TEL: 610-285-2006
FAX: 610-285-2007
timothy.e.dutt@monsanto.com

Michael Fernandez
Pew Initiative on Food and Biotechnology
TEL: 202-347-9044
FAX: 202-347-9047
Mfernandez@pewagbiotech.org

Sharie Fitzpatrick
Forage Genetics International
TEL: 608-786-2121
FAX: 608-786-2193
sfitzpatrick@foragegenetics.com

R. Warren Flint (Facilitator)
Five E's Unlimited
TEL: 202-232-4591
rwflint@eeeee.net

Crystal Fricker
Pure Seed Testing, Inc.
TEL: 503-651-2130
FAX: 503-651-2151
crystal@turf-seed.com

Ryan Goss
University of Nebraska–Lincoln
TEL: 402-472-1181
FAX: 402-472-8650
rgross@unlserve.unl.edu

Dean M. Graves
Chevy Chase Golf Club
TEL: 301-656-6323
FAX: 301-986-4653
gravesfour@aol.com

Teresa A. Gruber
CAST
TEL: 202-675-8333, ext 11
FAX: 202-675-8334
tgruber@cast-science.org

Subhash Gupta
USDA–APHIS–BRS
TEL: 301-734-8761
FAX: 301-734-8669
subhash.c.gupta@aphis.usda.gov

William K. Hallman (Cochair)
Rutgers University, Food Policy Institute
TEL: 732-932-1966, ext 3103
FAX: 732-932-9544
hallman@aesop.rutgers.edu

Deb Hamernik
USDA–CSREES
TEL: 202-401-4202
FAX: 202-401-1602
dhamernik@reeusda.gov

John Hammond
USDA–ARS, USNA, Floral & Nursery Plants Research Unit
TEL: 301-504-5313
FAX: 301-504-5096
hammondj@ba.ars.usda.gov

Bob Harriman
The Scotts Company
TEL: 937-644-7610
FAX: 937-644-7597
Bob.Harriman@Scotts.com

Robert Hedberg
Weed Science Society of America
TEL: 202-408-5388
FAX: 202-408-5385
robhedberg@erols.com

David Heron
USDA–APHIS–BRS
TEL: 301-734-5141
FAX: 310-734-8669
david.s.herson@aphis.usda.gov

Aimee Hill (Facilitator)
Hill Consulting
TEL: 703-532-9245
FAX: 703-532-9244
alhill@olg.com

Thomas K. Hodges
HybriGene, Inc./Purdue University
TEL: 410-745-9505
FAX: 410-745-0256
tkhodges@purdue.edu

Andrew Hopkins
Noble Foundation, Inc.
TEL: 580-224-6850
FAX: 580-224-6802
aahopkins@noble.org

Michael Horak
Monsanto Company
TEL: 314-694-3476
FAX: 314-694-8874
michael.j.horak@monsanto.com

Peter T. Jenkins
International Center for Technology Assessment
TEL: 202-547-9359
FAX: 202-547-9429
peterjenkins@icta.org

Margaret Jones
USDA–APHIS–BRS
TEL: 301-734-4880
FAX: 301-734-8669
margaret.j.jones@aphis.usda.gov

Wendelyn W. Jones
FDA–Center for Veterinary Medicine
TEL: 301-827-6978
FAX: 301-594-2298
wjones@cvm.fda.gov

Keith Karnok
University of Georgia
TEL: 706-542-0931
FAX: 706-542-0914
kkarnok@uga.edu

Albert Kausch
HybriGene, Inc.
TEL: 401-874-9900
FAX: 401-874-7124
albert@hybrigene.com

Mike Kelty
The Scotts Company
TEL: 937-644-7607
FAX: 937-644-7597
Mike.Kelty@Scotts.com

Mike Kenna (Cochair)
U.S. Golf Association
TEL: 405-743-3900
FAX: 405-743-3910
mkenna@usga.org

Tom Kirsch
NewEra Seed
TEL: 541-475-3843

Michael Kisielewski
National Research Council
TEL: 202-334-3069
FAX: 202-334-1978
mkisiele@nas.edu

Susan Koehler
USDA–APHIS–BRS
TEL: 301-734-4886
FAX: 301-734-8669
susan.m.koehler@aphis.usda.gov

Jeffrey Krans
Mississippi State University
TEL: 715-543-2114
FAX: 662-325-8742
jkrans@pss.msstate.edu

Lisa Lee
The Scotts Company
TEL: 937-644-7078
FAX: 937-644-7597
lisa.lee@scotts.com

Bruce MacBryde
USDA–APHIS Biotechnology Regulatory Service
TEL: 301-734-5787
FAX: 301-734-8669
bruce.macbryde@aphis.usda.gov

Carol Mallory-Smith
Oregon State University
TEL: 541-737-5883
FAX: 541-737-3407
Carol.Mallory-Smith
@oregonstate.edu

Betty H. Marose
University of Maryland
TEL: 301-405-3929
FAX: 301-314-9290
bm7@umail.umd.edu

Mark McCaslin
Forage Genetics International
TEL: 952-226-3930
FAX: 952-226-2931
mccaslin@forage-genetics.com

Rick Meilan
Oregon State University
TEL: 541-737-6097
FAX: 541-737-1393
Richard.Meilan@orst.edu

Virgil Meier
USDA–APHIS–BRS
TEL: 301-734-3363
FAX: 301-734-8669
Virgil.d.meier@aphis.usda.gov

Arthur Milberger
Turfgrass America
TEL: 979-245-2655
FAX: 979-245-1508
amilberger@turfgrassamerica.com

Kevin Morris
National Turfgrass Evaluation
Program
TEL: 301-504-5125
FAX: 301-504-5167
kmorris@ntep.org

Eric Nelson
The Scotts Company
TEL: 937-644-0011
FAX: 937-644-7597
eric.nelson@scotts.com

Tom Nickson
Monsanto Company
TEL: 314-694-2179
FAX: 314-694-8774
Thomas.Nickson@monsanto.com

Joe O'Grodnick
BASF
TEL: 919-547-2612
FAX: 919-547-2880
ogrodnj@basf-corp.com

Ronald L. Olson
NewEra Seed
TEL: 541-475-2661

Dawn W. Parks
ArborGen
TEL: 843-851-5077
FAX: 843-832-2164
dwparks@arborgen.com

Les Pearson
ArborGen
TEL: 843-851-4597
lxpears@arborgen.com

Leah Porter
CropLife America
TEL: 202-296-1585
FAX: 202-463-0474
LPORTER@CROPLIFEAMERICA.ORG

Bill Price
FDA–Center for Veterinary
Medicine
TEL: 301 827 6652
Bill.Price@fda.gov

Zac Reicher
Purdue University
TEL: 765-494-9737
FAX: 765-496-6335
zreicher@purdue.edu

Cindy Lynn Richard
CAST
TEL: 202-675-8333, ext 12
FAX: 202-675-8334
crichard@cast-science.org

Domingo C. Riego
Monsanto Company
TEL: 317-575-8769
FAX: 317-574-9157
domingo.c.riego@monsanto.com

Terrance Riordan
University of Nebraska/C5 Divi-
sion, CSSA
TEL: 402-472-1142
FAX: 402-472-7904
triordan@unl.edu

Guinn Roberts
USEPA/OSCP
TEL: 202-564-7464
FAX: 202-564-8502
Roberts.Guinnevere@epa.gov

Jamesine Rogers
USEPA
TEL: 202-564-3453
FAX: 202-564-9490
rogers.jamesine@epa.gov

John Rooney
Oklahoma State University
TEL: 405-377-5651
FAX: 405-377-5652
jfxroon@aol.com

Bill L. Rose
Tee-2–Green Corp.
TEL: 503-651-2130
FAX: 503-651-2351
bill@turf-seed.com

Robert Rose
USDA–APHIS
TEL: 301-734-4866
FAX: 301-734-8669
bob.I.rose@aphis.usda.gov

Craig Roseland
USDA–APHIS–BRS
TEL: 301-734-7935
FAX: 301-734-8669
craig.r.roseland@aphis.usda.gov

Mark Sagoff
Institute for Philosophy and Public
Policy
TEL: 301-405-4762
FAX: 301-314-9346
ms2@umail.umd.edu

Susan Samudio
J. R. Simplot/Jacklin Seed
TEL: 208-777-6152
FAX: 208-773-4846
ssamudio@simplot.com

Christiane Schmenk
The Scotts Company
TEL: 937-644-7606
FAX: 937-644-7225
chris.schmenk@scotts.com

Robert (Bob) C. Shearman
University of Nebraska–Lincoln
TEL: 402-472-0022
FAX: 402-472-8650
rshearmen1@unl.edu

Matt Shipp
Louisiana State University
TEL: 225-578-4568
FAX: 225-578-2257
mshipp1@lsu.edu

Jim Snow
USGA Green Section
TEL: 908-781-1074
FAX: 908-781-1736
jsnow@USGA.ORG

Carmen Soileau
USDA–APHIS–BRS
TEL: 301-734-5301
FAX: 301-734-8669
lene.c.soileau@usda.gov

Brian Steinwand
EPA, Biopesticide Division
TEL: 703-305-7973
FAX: 703-308-7026
steinwand.brian@epa.gov

John Stier
University of Wisconsin–Madison
TEL: 608-262-1490
FAX: 608-262-4743
jstier@facstaff.wisc.edu

Terry Stone
Monsanto Company
TEL: 636-737-6547
FAX: 636-737-5943
terry.b.stone@monsanto.com

Donald Suttner
Monsanto Company
TEL: 324-694-1213
FAX: 314-694-4940
donald.l.sutter@monsanto.com

Patricia Sweeney
Ohio State University
TEL: 614-292-8433
FAX: 614-292-2030
sweeney.4@osu.edu

Kathy Swords
J. R. Simplot Company
TEL: 208-327-3311
FAX: 208-327-3212
kathy.swords@simplot.com

Dennis Szuhay
EPA, Office of Pesticide Programs,
Registration Division
TEL: 703-305-6098
FAX: 703-308-1825
Szuhay.Dennis@epa.gov

Charles M. Taliaferro
Oklahoma State University
TEL: 405-744-9627
FAX: 405-744-5269
cmt@mail.pss.okstate.edu

Alan V. Tasker
USDA–APHIS–BRS
TEL: 301-734-5708
FAX: 301-734-8584
Alan.V.Tasker@usda.gov

Scott Thenell
S. Thenell Company
TEL: 925-933-1075
scottthenell@earthlink.net

Lane Tredway
North Carolina State University
TEL: 919-513-4820
FAX: 919-515-7716
lane_tredway@ncsu.edu

John Turner
USDA–APHIS, Biotechnology
Regulatory Services
TEL: 301-734-8365
FAX: 301-734-8669
john.t.turner@ahis.usda.gov

Kevin Turner
The Scotts Company
TEL: 503-792-3633
FAX: 503-792-3637
kevin.turner@scotts.com

Kai Umeda
University of Arizona Cooperative
Extension
TEL: 602-470-8086
FAX: 602-470-8092
kumeda@ag.arizona.edu

J. Bryan Unruh
University of Florida
TEL: 850-995-3720, ext 108
FAX: 850-995-3723
jbu@ufl.edu

Zengyu Wang
Samuel Roberts Noble Foundation
TEL: 580-224-6800
FAX: 580-224-6802
zywang@noble.org

Scott Warnke
USDA–ARS
TEL: 202-245-4570
FAX: 202-245-4579
warnkes@ars.usda.gov

Ken Waters
USDA
TEL: 301-734-8889

Tom Watschke
Pennsylvania State University
TEL: 814-863-7644
FAX: 814-863-7043
tlw3@psu.edu
Norm Weisner
4B Farms

James L. White
Visiting Scientist from USDA to
National Invasive Species Council
TEL: 202-354-1882
FAX: 202-371-1751
James_White@ios.doi.gov

D. W. Williams
University of Kentucky
TEL: 859-257-2715
FAX: 859-323-1952
dwilliam@uky.edu

Joseph Wipff
Pure Seed Testing, Inc.
TEL: 503-651-2130
FAX: 503-651-2351
joseph@turf-seed.com

LaReesa Wolfenbarger
Virginia Tech/Information Systems
for Biotechnology
TEL: 402-238-2723
FAX: 540-231-9070
lwolfenb@vt.edu

Christiane Wozinak
USEPA, Biopesticides and Pollution Prevention Division
TEL: 703-605-0513
FAX: 703-308-7026
wozniak.chris@epa.gov

Fred Yelverton
North Carolina State University
TEL: 919-515-5639
FAX: 919-515-5315
fred_yelverton@ncsu.edu

Floyd A. Yoder, Jr.
New Jersey Dept. of Agriculture, Division of Plant Industry
TEL: 609-292-5443
FAX: 609-292-4710
Floyd.Yoder@ag.state.nj.us

Appendix D: Workshop Agenda

Biotechnology-derived, Perennial Turf and Forage Grasses: Criteria for Evaluation

Thursday, January 9, 2003

Open to the public (advanced registration necessary, no admittance fee)

(Meeting Room: General Sessions on Thursday will be held in the Baltimore Room)

10:00 a.m. Introduction
Teresa A. Gruber, Ph.D., J.D.—Council for Agricultural Science and Technology

10:10 a.m. Issues in Turfgrasses and Potential Biotech Solutions
Michael P. Kenna, Ph.D.—U.S. Golf Association, Green Section Research

10:30 a.m. Issues in Forage Grasses and Potential Biotech Solutions
Andrew Hopkins, Ph.D.—Noble Foundation

10:50 a.m. USDA–APHIS' Role in the Regulation of Biotechnology-derived Turf and Forage Grasses
John Turner, Ph.D.—USDA, Animal and Plant Health Inspection Service

11:20 a.m. EPA Perspective on Biotechnology-derived Turf and Forage Grasses
Dennis Szuhay, Ph.D.—U.S. Environmental Protection Agency

11:40 a.m. Questions and Discussion of Morning Presentations
Moderator: Teresa Gruber, Ph.D., J.D.

12:00 noon Lunch with speakers, steering committee, writing committee. and staff
(Lunch in the Annapolis room)

1:30 p.m. Open Public Comment
Moderator:
William K. Hallman, Ph.D.—Rutgers University, Food Policy Institute
Providing Comments:

1. Bill Price—FDA, Center for Veterinary Medicine
2. Mark McCaslin—Forage Genetics International
3. Eric Nelson—The Scotts Company
4. Thomas Nickson—Monsanto Company
5. Terrance Riordan—University of Nebraska and C5 Division, CSSA
6. Bill L. Rose—Tee-2-Green Corp.
7. Crystal Fricker—Pure Seed Testing, Inc.
8. Joseph Duich—Penn State University
9. Douglas J. Cattani—Graminae Consulting and Research
10. Prasanta C. Bhowmik—University of Massachusetts, Amherst
11. Peter T. Jenkins—International Center for Technology Assessment

2:30 p.m. Turf and Forage Grass Biology and Management
Keith Karnok, Ph.D.—University of Georgia
Michael Casler, Ph.D.—U.S. Dairy Forage Research Center

3:10 p.m. Turf and Forage Grass Breeding
Charles Taliaferro, Ph.D.—Oklahoma State University

3:30 p.m. Questions and Discussion on Grass Biology, Management, and Breeding
Moderator: J. Bryan Unruh, Ph. D.—University of Florida

3:50 p.m. Break provided for all participants outside meeting room

4:10 p.m. **Weed Science/Invasive Species Concerns: A Weed Ecologist's Review**
Carol Mallory-Smith, Ph.D.—Oregon State University

4:30 p.m. **Weed Science/Invasive Species Concerns: A Weed Scientist's Review**
John Stier, Ph.D.—University of Wisconsin

4:50 p.m. **Questions and Discussion**

5:30 p.m. **Dinner with speakers, steering committee, writing committee, and staff**
(Dinner in Columbia room)

7:00–10:00 p.m. Breakout Session I—Seed Characteristics
(Meeting Room: Constellation E or F)

1. Introductions
2. Discussion: What Are the Big Picture Issues Related to Seed Characteristics of Biotechnology-derived, Perennial Turf and Forage Grasses?
3. Discussion of Posted Questions
4. Wrap-up and Review of Breakout Session I Discussions

Friday, January 10, 2003

Open to the public (advanced registration necessary, no admittance fee)

(Meeting Room: General Sessions on Friday will be held in Constellation E)

8:00 a.m. **Open public comment—Written input welcome through February 15, 2003**
Moderator:
John Rooney, Ph.D.—Oklahoma State University
Providing Comments:

1. Leah A. Brilman—Seed Research of Oregon
2. Jeffrey Krans—Mississippi State University
3. Albert Kausch—HybriGene, Inc.
4. Donald Suttner—Monsanto Company
5. Rick Meilan—Forest Science Department, Oregon State University
6. Thomas K. Hodges—HybriGene, Inc.–Purdue University

8:30 a.m. **Breakout Session II—Vegetative Characteristics**
(Meeting Room: Constellation E or F)

1. Discussion: What Are the Big Picture Issues Related to Vegetative Characteristics of Biotechnology-derived, Perennial Turf and Forage Grasses
2. Discussion of Posted Questions
3. Wrap-up and Review of Breakout Session II Discussions

11:15 a.m. **Lunch with speakers, steering committee, writing committee, and staff**
(Lunch in the Columbia Room)

12:15 p.m. **Breakout Session III—Flowering Characteristics**

1. Discussion: What Are the Big Picture Issues Related to Flowering Characteristics of Biotechnology-derived, Perennial Turf and Forage Grasses?
2. Discussion of Posted Questions
3. Wrap-up and Review of Breakout Session III Discussions

3:00 p.m. **Break available for all participants outside meeting room**

3:15 p.m. **Entire group reconvene for breakout session reports and discussion**

4:30 p.m. **Workshop adjourns**

Dinner with speakers, steering committee, writing committee, and staff
(Time and location to be announced)

Saturday, January 11, 2003

(Meeting Room: Columbia Room)

8 a.m.–12:00 noon Writing Team Meeting (open to writing team, steering committee, and staff)

Appendix E: Abbreviations and Acronyms

ALS	acetolactate synthase
APHIS	Animal and Plant Health Inspection Service
BD	biotechnology-derived
CAD	cinnamyl alcohol dehydrogenase
CAST	Council for Agricultural Science and Technology
CBF	C-repeat binding factor
CFIA	Canadian Food Inspection Agency
cm	centimeter
COMT	caffeic acid O-methyl transferase
DREB	dehydration responsive element binding protein
EPA	U.S. Environmental Protection Agency
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
F_1	first filial generation
GM	genetically modified
m	meter
mt1D	mannitol 1-phosphate dehydrogenase
N	nitrogen
P	phosphorus
PAP	Pokeweed Antiviral Protein
PEG	polyethylene glycol
PVP	plant variety protection
PVPA	Plant Variety Protection Act
USDA	U.S. Department of Agriculture
VA	vesicular-arbuscular

Appendix F: Glossary

Abiotic factor. Nonliving components (e.g., temperature, moisture, nutrient deficiency, light) that affect the environment of living organisms.

Additivity. The process of adding a substance in small amounts to something else to improve, strengthen, or otherwise alter it.

Adventitious root. A root growing in an unusual location (e.g., from a stem).

Alkaloid. Any of numerous, usually colorless, complex and bitter organic bases containing nitrogen and usually oxygen that occur in seed plants.

Allele. Any of the alternative forms of a gene that may occur at a given position in a chromosome.

Anthesis. The period during which a flower is fully open and functional.

Antisense suppression. Suppression of a complementary sequence to a segment of genetic material.

Apical meristem. A meristem at the tip of a plant root or shoot that causes the root or shoot to increase in length.

Apomixis. Reproduction involving specialized generative tissues but not dependent on fertilization.

Assay. Analysis to determine the presence, absence, or quantity of one or more components.

Auricle. An angular or ear-shaped lobe, process, or appendage, especially at the base of an organ.

Autotetraploid. A strain whose chromosome complement consists of four copies of a single genome resulting from doubling of an ancestral chromosome complement.

Autotrophic. Of or relating to organisms that can make complex organic nutritive compounds from simple inorganic substances.

Auxin-type pesticide. A pesticide that causes the elongation of plant cells in shoots to control plant growth and development.

Axenic culture. Free from living organisms of any kind other than those stated or implied.

Axillary bud. A bud situated in or rising from the upper angle between a lateral organ and the stem that bears it.

Axillary meristem. Type of meristem in buds at leaf axils.

Biolistics. The process of shooting a gene into plant cells and subsequently growing a plant from the cells containing the transgene; also referred to as particle acceleration.

Biota. The flora and fauna of a region.

Biotechnology. The tools and technology used to make products from biological systems (e.g., cheese making), to carry out processes using biological substances (e.g., enzyme-based processing such as wine making), or to modify biological systems in order to improve performance or produce biomaterials (e.g., breeding, tissue culture, cloning, transgenics).

Biotic factor. A factor caused by a living organism (e.g., fungi, bacteria, parasites, competitors [weeds], insects).

Callus. A thickening of or a hard thickened area on skin or bark.

Caryopsis. A small, one-seeded fruit that remains closed at maturity and has a thin membranous ovary wall fused to the seed-coat (e.g., wheat, barley, certain grasses).

Chloroplast. An organelle that contains chlorophyll and is the site of photosynthesis.

Coat protein. Protective shell around viral DNA or RNA made of one to several layers of protein.

Colorimetry. The process of determining and specifying colors.

Congeners. Plants or animals of the same taxonomic genus.

Conspecific. Of the same species.

Corm. A rounded, thick, modified underground stem base bearing membranous or scaly leaves and buds and acting as a vegetative reproductive structure.

Cross-resistance. Tolerance to a normally toxic substance acquired by exposure to a related substance rather than direct exposure to a toxin.

Culm. The jointed stem of a grass, usually hollow except at the often-swollen nodes, and usually herbaceous.

Cultivar. An organism of a kind originating and persistent under cultivation; term synonymous with variety.

Determinate plant. A plant with definite limits, characterized by sequential flowering from the central or uppermost bud to the lateral or subordinate buds.

Dioecious. Having male reproductive structures in one individual and female reproductive structures in another individual.

Diploid. Having the basic chromosome number doubled.

DNA technology. The means by which new genes are incorporated into plants using a range of molecular techniques.

Domestication. To adapt a plant to life in intimate association with and to the advantage of humans.

Dominance. The property of one of a pair of alleles or traits that suppresses expression of the other in the heterozygous condition.

Down regulated. Decreased expression of genes.

Drill row. Seed established at various soil depths within a row.

Ectomycorrhizal. The state in which mycorrhiza grow on the surface roots of plants.

Electroporation. The use of strong, brief pulses of electric current to create temporary holes in cell membranes, allowing the introduction of DNA.

Endogenous. Growing or produced by growth from deep tissue within the organism or system.

Endomycorrhizae. A form of mycorrhiza in which fungi grow on the surface of roots and invade all cells of the root cortex.

Endophyte. A plant living within another plant.

Endophytic fungi. Fungi living within another plant.

Endophytic mycelium. Mycelium living within another plant.

Endosperm. A nutritive tissue in seed plants formed within the embryo sac.

Ergot. The black or dark purple sclerotium of fungi that occurs as a club-shaped body replacing the seed of grass.

Event. The integration of a transgene into a specific location in the DNA of the recipient plant.

Exposure. In relation to BD plants, exposure represents the probability that the hazard will occur. *See also* **Hazard.**

Facultative apomixis. A condition in which both sexual and apomictic reproduction occur within individual plants or among plants within a population.

Familiarity. In BD crops, familiarity encompasses the existing knowledge and experience with a specific crop plant, the BD trait or phenotype, the ecosystem in which the plant will be used, and interactions among these elements.

Feral. Wild, rather than domesticated or cultivated.

Floral induction. Initiation of the production of flowers, possibly stimulated by the hormone florigen.

Floret. One of the small flowers forming the head of a composite plant.

Fructan. A polysaccharide whose constituent monosaccharides are fructoses.

Gene flow. The exchange of genetic traits between populations by movement of individuals, gametes, or spores, involving the spread of new gene variants among different populations through dispersal. Gene flow and mutation are, therefore, the only means by which new genetic factors may be introduced into a population.

Gene stacking. *See* **Stacked genes**.

Germplasm pool. Germ cells and their precursors serving as the bearers of heredity, fundamentally independent of other cells.

Grass tetany. A disease of livestock caused by magnesium deficiency, occurring when there is a change from indoor feeding to outdoor grazing.

Hand emasculation. In plants, the removal of male flowers or anthers to prevent self-pollination.

Hazard. In relation to BD plants, the severity of an unwanted environmental change resulting from release of the BD plant. *See also* **Exposure.**

Hemizygous plant. Having or being characterized by one or more genes with no allelic counterparts.

Herbaceous. Having characteristics of an herb.

Herbicide resistance. *See* **Herbicide tolerance**.

Herbicide tolerance. The inherent or acquired ability of a plant to survive and reproduce after exposure to a dose of herbicide that normally would be lethal to the targeted plants.

Herbivory. Feeding on plants.

Heritable variation. Variations in what is inherited from one's ancestors.

Heterosis. The marked vigor or capacity for growth often exhibited by crossbred animals or plants.

Heterozygous. Having the two alleles at corresponding loci on homologous chromosomes different for one or more loci.

Homozygous. Having the two genes at corresponding loci on homologous chromosomes identical for one or more loci.

Inbreeding depression. A condition whereby plants lose vigor or display other anomalies, sometimes when closely related perennial grasses are interbred.

Indeterminate plant/grass/flowering. Characterized by sequential flowering from the lateral or basal buds to the central or uppermost buds.

Induction. *See* **Floral induction**.

Inflorescence. The flowering part of a plant and especially the mode of its development and arrangement of flowers on an axis.

Initiation stimuli. Temperature, light, moisture, or other environmental factors that induce the grass plant to begin the flowering process.

Intercalary meristem. A region of cell division at or near the collar.

Intercrossing. An instance of or a product of crossbreeding.

Internode. An interval or part between two nodes of a stem.

Interspecific crossing. Breeding between two species.

Interspecific hybridization event. Arising or occurring between species.

Intervarietal. Between fields.

Intraspecific crossing. Breeding among its own species.

Intravarietal. Within a single field.

Introgression. The introduction of one gene from one gene complex into another.

Invasiveness. Spreading aggressively from the original site of planting.

Isolation distance. A calculated or prescribed distance sufficient to prevent the genetic material of one plant or crop from unintentionally fertilizing other plants or crops.

Isoline. A line on a map or chart along which there is a constant value.

Lamina. The extended part of a foliage leaf; a blade; the leafy portion of a frond.

Lemma. The lower of the two bracts enclosing the flower in the spikelet of grasses.

Lignin. A class of compounds found in plant cell walls, important in providing structural support and disease resistance to plants.

Ligule. A thin appendage of a foliage leaf and especially on a blade of grass.

Linkage drag. Unwanted genes associated with a desirable gene that lower the performance of the plant.

Mass selection. A form of selection in which individual plants are selected and the next generation propagated from the aggregate of their seed.

Meiosis. The cellular process that results in the number of chromosomes in gamete-producing cells being reduced to one half and that involves a reduction division in which one of each pair of homologous chromosomes passes to each daughter cell and a mitotic division.

Meristem. A formative plant tissue made up of undifferentiated small cells capable of dividing indefinitely and giving rise to similar cells or to cells that differentiate to produce the definitive tissues and organs.

Metabolomics. Concentrations of metabolites that are the direct reflection of metabolism. By measuring changes in metabolite concentrations, the full range of biochemical effects can be determined; can be used to diagnose or predict disease and stratify populations by their specific metabolism.

Molecular construct. These are laboratory-designed plasmids that contain an interesting gene for insertion into plant genomes. A plasmid is a small, circular double-stranded DNA molecule, existing inside a host such as a bacterium, which replicates independently of the host genetic material. Because of their small size, plasmids can be easily manipulated in the laboratory. They are the primary vehicle used to isolate, clone, express and otherwise manipulate genes and DNA sequences of interest.

Monoecious. Having male and female sex organs on the same plant.

Morphology. A branch of biology that deals with the form and structure of animals and plants..

Multiple resistance. More than one resistance characteristic to a disease or insect pest.

Mutagenesis. The introduction into a gene of an alteration that results in a change in the structure or function of the gene product.

Mycorrhizae. The symbiotic association of the mycelium of a fungus with the roots of a seed plant.

Node. The often-swollen or otherwise modified point on a stem at which a leaf or leaves are attached.

Null segregant. Progeny from parent plants closely related to the transformed parents used to develop the BD perennial grass variety.

Obligate apomixis. A breeding system in which organisms reproduce asexually without meiosis or formation of gametes.

Osmoregulation. The regulation of osmotic pressure in the body of a living organism.

Outcrossing. The transfer of a given gene or genes from a domesticated organism to a wild-type (plant relative).

Palea. The upper bract that with the lemma encloses the flower in grasses.

Panicle. A pyramidal, loosely branched flower cluster.

Perennating organ. An organ that lives more than one growing season.

Phenolic compound. A compound containing a hydroxyl group bonded directly to a benzene ring.

Phenotype. The observable characteristics of an organism produced by the interaction of the genotype and the environment.

Photoperiod. A reoccurring cycle of light and dark periods of constant length.

Phytomer. Part of a grass stem that includes a node, internode, and leaf.

Pistillate plant. Having pistils but no stamen.

Plant genomics. The branch of genetics that studies organisms in terms of their genomes (their full DNA sequences).

Plasmid. A small, self-replicating piece of DNA found outside the chromosome. Plasmids are the principal tools for inserting new genetics information into microorganisms or plants.

Ploidy level. Degree of repetition of the basic number of chromosomes.

Plugging. A generic term that refers to planting of a vegetative section (plug) of a stoloniferous and/or rhizomatous turfgrass.

Polyploidy. Having a chromosome number that is a multiple greater than two of the monoploid number.

Proteomics. Identification, characterization, and quantification of all proteins involved in a particular pathway, organelle, cell, tissue, organ, or organism that can be studied together to provide accurate and comprehensive data about that system.

Protoplast. A plant cell that has had its cell wall removed.

Raceme. A simple inflorescence in which the flowers are borne on short stalks of about equal length at equal distances along an elongated axis and open in succession toward the apex.

Rhizobia. Any of a genus of small, heterotrophic soil bacteria capable of forming symbiotic nodules on the roots of leguminous plants and thereby becoming bacteriods that fix atmospheric nitrogen.

Rhizome. A somewhat elongated, horizontal subterranean plant stem that often is thickened by deposits of reserve food material, produces shoots above and roots below, and is distinguished from a true root in possessing buds, nodes, and usually scalelike leaves.

Rhizosphere. Soil that surrounds and is influenced by the roots of a plant.

Risk assessment. The scientific method that assesses the probability that a harmful effect will occur.

Scarification. The process of cutting or softening the wall of a hard seed to hasten germination.

Segregation ratios. The proportion of progeny of a particular genotype or phenotype from actual matings of specific genotypes.

Selectable markers. An easily observed trait linked to one that may be more difficult to measure; used to screen the population for the desirable linked trait.

Senesce. A plant or plant part going from full maturity to death; to become old.

Sense suppression. A process whereby multiple copies of a gene are introduced into plant chromosomes.

Siliceous dentation. Leaf margins that are rough or sawlike, making them less palatable to foraging animals.

Somaclonal variation. In tissue culture, individual plant cells induced to produce whole plants that may provide variation among the whole plants for certain characteristics.

Speciation. The process of biological species formation.

Spike. An elongated inflorescence similar to a raceme but having the flowers attached directly by the base on the main axis (e.g., common plantain).

Sprigging. To propagate (a grass) by means of stolons or small divisions.

Stacked genes. Breeding or engineering two or more genes for different traits into one cultivar or hybrid; currently, stacked cotton is both insect resistant and herbicide resistant.

Staminate plant. A plant that has or produces stamens but no pistils.

Stolon. A horizontal branch from the base of a plant that produces new plants from buds at its tip or nodes; also called a runner.

Stressor. Stimulants that cause stress.

Sward. A portion of ground covered with grass.

Symbiont. An organism living in symbiosis, usually the smaller member of a symbiotic pair.

Sympatry. Two or more closely related species having coincident or overlapping ranges of distribution but not interbreeding.

Symplast. A continuous network of interconnected plant cell protoplasts.

Taxonomy. Orderly classification of plants and animals according to their presumed natural relationships.

Tiller. A twig or shoot from the base of a plant or from the axils of its lower leaves.

Transgene. A gene that is or has been introduced into the genome of another organism.

Transgenic. Containing genes altered by insertion of DNA from an unrelated organism. Taking genes from one species and inserting them into another species to get that trait expressed in the offspring. The term "GMO" (genetically modified organism) often is used mistakenly when "transgenic" or "biotechnology-derived" products are the intended reference.

Translocation. Movement of a nutrient or chemical from one part of the plant to another.

Trichome. A filamentous outgrowth, especially an epidermal hair structure on a plant.

Tussock. A compact tuft of grass or sedge.

Unconscious selection. A form of selection resulting from the attempt to preserve the most valued and destroy the least valued offspring, without intent of altering the breed itself.

Vernalization. The act or process of hastening the flowering and fruiting of plants by treating seed, bulbs, or seedlings to induce a shortening of the vegetative period.

Whiskers transformation. A process similar to biolistics, except that plant cells are stirred in a liquid containing silicon carbide fibers coated with the transgene, facilitating insertion of the DNA into plant cells like small needles.

Woody. Forming stems that mature to wood.

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