The Science of Gene flow in Agriculture and its Role in Co-existence

Proceedings

Washington, DC
September 7-8, 2011

United States Department of Agriculture
National Institute of Food and Agriculture
UC DAVIS
DEPARTMENT OF PLANT SCIENCES
College of Agricultural and Environmental Sciences
SEED BIOTECHNOLOGY CENTER

The Science of Gene Flow in agriculture and its Role in Co-existence, Washington, DC

SEPTEMBER 7th, 2011

	Topic	**Speaker, Institution**
7:30 AM	Coffee and light refreshments	
8:30 AM	Welcome and meeting goals	Allen Van Deynze, UC Davis
8:40 AM	Opening remarks	Catherine Woteki, USDA Chief Scientist, Undersecretary for Research, Education and Economics
Impact of Gene Flow on Agriculture (Moderator: Allen Van Deynze)		
8:55 AM	Economic impact of gene flow	Nicholas Kalaitzandonakes, U of Missouri
9:20 AM	Maintaining seed purity in the seed trade industry	Ric Dunkle, American Seed Trade Association
9:45 AM	The implications of gene flow on organic farming	Mark Lipson, OSEC-USDA Organic and Sustainable Ag Policy
10:10 AM	**BREAK**	
10:30 AM	Importance of gene flow to germplasm conservation and development	Stephanie Greene, USDA/ARS, Washington
10:55 AM	The potential impact of gene flow mitigation on agriculture	Kent Bradford, UC Davis
11:20 AM	Panel discussion: Impact of Gene flow on Agriculture	
Gene Flow In the Environment (Moderator: Neal Stweart)		
11:45 AM	Outcrossing to wild relatives	Alison Snow, Ohio State
12:10 PM	**LUNCH**	
1:10 PM	Potential for persistence of genes in the environment	Joe DiTomaso, UC Davis
1:35 PM	Movement of genes in grasses	Carol Mallory-Smith, Oregon State
2:00 PM	Movement of honeybees in alfala	James Hagler, USDA/ARS
2:25 PM	Gene flow in alfalfa	Larry Teuber, UC Davis
2:50 PM	Challenges of organic alfalfa seed production	Ray Johnson, TopNotch Seed
3:15 PM	**BREAK**	
3:35 PM	Gene flow between feral and cultivated alfalfa populations	Rene Van Acker, University of Guelph
4:00 PM	Panel discussion: Gene flow in the environment	
Gene Flow Mitigation Strategies (Moderator: Kent Bradford)		
4:25 PM	Overview of male sterility strategies	Neal Stewart, U of Tennessee
4:50 PM	Non-transgenic cross incompatibility systems in maize	Matt Evans, Stanford University
5:15 PM	Transgenic flower sterility strategies	Zhongchi Liu, U of Maryland
6:00 PM	**Social and discussions-Whitten Patio** *(no host bar)*	

SEPTEMBER 8th, 2011

7:30 AM	Coffee and light refreshments	
8:30 AM	Workshop announcements	Allen Van Deynze
Gene Flow Mitigation Strategies (continued)		
8:35 AM	Male sterility in hybrid systems	Marc Albertsen, Pioneer Hi-Bred
9:00 AM	Transgenic seed sterility strategies	Mike Portereiko, Ceres
9:25 AM	Reversible male sterilty using chloplast transformation	Henry Daniell, U of Central Florida
9:50 AM	**BREAK**	
10:15 AM	Transgenic containment in trees	Steve Strauss, Oregon State
10:40 AM	Panel discussion: Gene flow mitigation strategies	
11:05 AM	General discussion-Impact, gene flow and mitigation strategies	Allen Van Deynze
11:30 AM	**Close**	

The Science of Gene Flow in Agriculture and its Role in Co-existence

September 7-8, 2011
Washington, DC

Dr. Catherine Woteki is Under Secretary for USDA's Research, Education, and Economics (REE) mission area, and the Department's Chief Scientist. Before joining USDA, Dr. Woteki served as Global Director of Scientific Affairs for Mars, Incorporated, where she managed the company's scientific policy and research on matters of health, nutrition, and food safety. From 2002-2005, she was Dean of Agriculture and Professor of Human Nutrition at Iowa State University. Dr. Woteki served as the first Under Secretary for Food Safety at the U.S. Department of Agriculture (USDA) from 1997-2001, where she oversaw U.S. Government food safety policy development and USDA's continuity of operations planning. Dr. Woteki also served as the Deputy Under Secretary for REE at USDA in 1996. Prior to going to USDA, Dr. Woteki served in the White House Office of Science and Technology Policy as Deputy Associate Director for Science from 1994-1996. Dr. Woteki has also held positions in the National Center for Health Statistics of the U.S. Department of Health and Human Services (1983-1990), the Human Nutrition Information Service at USDA (1981-1983), and as Director of the Food and Nutrition Board of the Institute of Medicine at the National Academy of Sciences (1990-1993). In 1999, Dr. Woteki was elected to the Institute of Medicine of the National Academy of Sciences, where she has chaired the Food and Nutrition Board (2003-2005). She received her M.S. and Ph.D. in Human Nutrition from Virginia Polytechnic Institute and State University (1974). Dr. Woteki received her B.S. in Biology and Chemistry from Mary Washington College (1969).

Marc Albertsen is a Senior Research Fellow in the Ag Biotech group at Pioneer Hi-Bred Int., Inc. in Johnston, Iowa. He has overall responsibility for the development of corn that can meet the abiotic challenges of the next decade through changes in plant architecture, increasing cold and frost tolerance, and developing new approaches to hybrid production. Emphasis is primarily on corn, but applications to other crops are exploited as the opportunities occur. Previously, he was responsible for leading the Ag Traits Discovery and Validation Department in development of crops with improved drought tolerance, enhanced nitrogen use efficiency and enhanced yield. The department was also responsible for developing and delivering genetics-based technology to enhance the production of hybrid maize and for applying this technology to produce hybrids in other crops. Marc also is involved in philanthropic projects involving public-private partnerships. He is the PI for the Africa Biofortified Sorghum (ABS) project, and he is the Pioneer lead for the Improved Maize for African Soils (IMAS) project. Marc began his career at Pioneer Hi-Bred in 1981. Although his early career focused on working with cytoplasmic male sterility (CMS), he became responsible for coordinating the development of genetically engineered male fertility control systems that could be used to produce maize hybrids more efficiently than CMS. In 1997, he was named as a Pioneer Research Fellow. He became the Research Director for the Agronomic Traits department in 2001. He was named as a Senior Research Fellow in 2009. In 2008 he was recognized as the Iowa Inventor of the Year by the Iowa Intellectual Property Law Association. Marc holds a doctorate in Plant Breeding and Genetics from the University of Minnesota, a master’s degree in Plant Breeding and Cytogenetics from Iowa State University, and a bachelor’s degree in Botany from Iowa State University. He pursued postdoctoral studies at Iowa State University on the genetics and cytogenetics of nuclear and cytoplasmic color mutants in soybeans before joining Pioneer in 1981.

The Science of Gene Flow in Agriculture and its Role in Co-existence

September 7-8, 2011
Washington, DC

Kent J. Bradford is a Professor in the Department of Plant Sciences at the University of California, Davis. Originally from Texas, he earned his B.S. in Biochemistry and M.S. in Horticulture from Michigan State University and his Ph.D. in Plant Physiology from the University of California, Davis. After a postdoctoral fellowship at the Australian National University, he joined the faculty at UC Davis in 1982 and served as the Chair of the Department of Vegetable Crops from 1993 to 1998. In 1999 he founded the UC Davis Seed Biotechnology Center and continues to serve as its director. He teaches University *and Extension courses on seed biology and production and on biotechnology, ethics and* philosophy of science. He received a Fulbright Scholar award in 1999 and the career Seed Science Award from the Crop Science Society of America in 2002. He was elected a Fellow of the American Association for the Advancement of Science in 2003 and received the faculty Award of Distinction from the College of Agricultural and Environmental Sciences at UC Davis in 2007. Dr. Bradford's research has spanned diverse areas of seed science from seed germination and conservation to mathematical modeling and molecular biology. He has published over 150 peer-reviewed research and extension articles and book chapters and co-edited three books on seed biology. He is currently co-authoring the 3rd edition of *Seeds: Physiology of Development and Germination*.

Dr. Henry Daniell is a Pegasus Professor and the University Board of Trustee Chair in the College of Medicine, University of Central Florida (UCF). He is the Fellow of the American Association for the Advancement of Science and a member of the oldest (Italian) National Academy of Sciences. Recently, he received an American Diabetes Association Award, Bayer Hemophilia global award and Bill and Melinda Gates Foundation Award for his contributions on oral delivery of plant-made therapeutic vaccines.. He is recognized for pioneering chloroplast genetic engineering concept to introduce transgenes that are maternally inherited and avoid pollen transmission. He advanced this new platform to produce low cost enzymes for biofuels, vaccines and biopharmaceuticals and confer valuable agronomic traits to plants including herbicide/insect/disease resistance or tolerance to drought/salt or heavy metals. His laboratory is a global leader in chloroplast genomics and has determined the complete chloroplast genome sequences of more than thirty crop plants and trees. His research has been featured on the cover of several high impact scientific journals (Nature Biotechnology, Plant Cell, Plant Physiology, Plant Biotechnology Journal, etc), and ranked by Nature Biotechnology among the top ten inventions of the last decade and among Biomed Central's Hot 100 authors in the world. His research has been featured often in the public press including Discovery Channel, Voice of America, CNN, BBC, several other global networks, Paul Harvey, Jay Leno, New York Times and Scientific American. For more details on his research visit http://daniell.ucf.edu

The Science of Gene Flow in Agriculture and its Role in Co-existence

September 7-8, 2011
Washington, DC

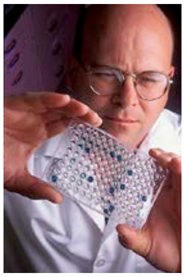
Dr. DiTomaso received his Ph.D. in Botany from the University of California, Davis in 1986. He was on the faculty at Cornell University until 1995 when he was appointed a Cooperative Extension Specialist in the Department of Plant Sciences at UC Davis. Dr. DiTomaso primarily conducts research on the biology, ecology and management of invasive plants and has published extensively in this area. He is also the author of three books on weeds. He served as President for the nonprofit California Invasive Plant Council and President of the Western Society of Weed Science. He is the director of the UC Weed Research and Information Center also serves on the National and the California Invasive Species Advisory Committee. He chaired the National Invasive Species Advisory Committee task team that developed federal guidelines proposed to reduce the risk of biofuel species becoming invasive. Dr. DiTomaso is the first editor of the new journal entitled Invasive Plant Science and Management. He is the recipient of the Outstanding Weed Scientist award by the Western Society of Weed Science, the Outstanding Extension award by the Weed Science Society of America, and he is a fellow in the Weed Science Society of America.

Ric Dunkle joined ASTA in January, 2008 after a 35 year career in state and Federal government. His career began in California in 1973 with the California Department of Food and Agriculture as a field entomologist. Over the span of 7 years he established CDFA's biological control, pest management, and environmental; monitoring programs. In 1981 he began his career with USDA, serving in the Secretary's Office of Environmental Quality. In 1983 he transferred to the USDA Agricultural Service where he held positions in research, management, and executive leadership. In 1999 he became Deputy Administrator of APHIS Plant Protection and Quarantine until his retirement in 2008. He holds a Bachelor of Science degree and a Ph D from the University of California, Davis. With ASTA, Ric handles the phytosanitary issues associated with seed movement and coordinates issues related to seed quality, testing, and research.

Matthew Evans received his PhD in Cell and Molecular Biology at the University of Pennsylvania with R. Scott Poethig, working on vegetative maturation in maize. He then received a NSF post-doctoral fellowship in plant biology to work with Jerry Kermicle at the University of Wisconsin-Madison. In 2001, Dr. Evans joined the Department of Plant Biology in Stanford, California as an adjunct staff member. Since then he has worked on the genetics and genomics of maize gametophyte function and also on pollen pistil interactions and cross incompatibility in *Zea*. In addition to maintaining a research program Dr. Evans also supervises an outreach program in collaboration with the Stanford University Science in Service program to train undergraduates as mentors and provide biology laboratory exercises to high school students.

The Science of Gene Flow in Agriculture and its Role in Co-existence
September 7-8, 2011
Washington, DC

Stephanie Greene has been working in the USDA National Plant Germplasm System since 1992. Since 1994, she has been the germplasm curator for the U.S. alfalfa and clover collections, which contain over 15,000 accessions, representing wild species, land races, obsolete and current cultivars and genetic stocks. In addition to collecting seed in locations such as Russian, Kazakhstan and Ukraine, she regenerates over 250 accessions each year in Prosser, WA. Seed from the collection is distributed worldwide. Dr. Greene also chairs the Crop Germplasm Committee (CGC) for alfalfa and serves as an ex officio on the Clover and Special Purpose Legumes CGC. She holds a Bachelor of Science and Masters of Science from the University of Idaho, and a Ph D from Kansas State University.



James Hagler is a Research Entomologist with the US Arid Land Agricultural Research Center in Maricopa, Arizona. He received his BS and MS from New Mexico State University and his PhD from The University of Arizona. His primary research areas are biological control, insect dispersal, and insect behavior. Dr. Hagler is considered an authority among biological control researchers in using molecular gut content analyses to evaluate the efficacy of predaceous natural enemies. He pioneered the “protein marking” method for use in area-wide mark-release-recapture and mark-capture type dispersal studies and he regularly mentors students and colleagues in the use of such methods. James has authored over 75 peer-reviewed publications.

Ray Johnson is a farmer and seed producer in the Imperial Valley of California, one of the premiere alfalfa hay- and seed-producing regions in the world. He owns Claborn Hay Company and is an owner of Top Notch Seed Company. Ray is chairman of the California Alfalfa Seed Research Board, past president of California Crop Improvement Association Board, and serves on the boards of the National Alfalfa & Forage Alliance (NAFA) and Western Alfalfa Seed Growers Association. Ray is a graduate of the California Ag Leadership Program. He was born and raised in the Imperial Valley and makes his home in Holtville, California with his wife Molly.

The Science of Gene Flow in Agriculture and its Role in Co-existence

September 7-8, 2011
Washington, DC

Nicholas Kalaitzandonakes is the MSMC endowed professor of agribusiness strategy and the director of the Economics and Management of Agrobiotechnology Center (EMAC) at the University of Missouri-Columbia. His research, teaching and outreach focus on the economics and policy of agrifood biotechnology and other agribusiness innovations. Over the past five years, Dr. Kalaitzandonakes has authored articles on the economic and environmental impacts of biotechnology; its impacts on the structure of the agrifood supply chain; the evolution of the agrifood biotechnology industry; the impact of biotechnology on international trade, and issues of biotechnology public acceptance and regulation.

He is the editor of AgBioForum, and has been an editorial board member for several other academic journals. He is also a member of scientific advising boards for startups, venture capital companies and national research centers involved in the life sciences and has been a member of the US Secretary of Agriculture Advisory Committee on Biotechnology and 21st Century Agriculture (AC21) and the Missouri Governor's Advisory Council for Plant Biotechnology.

Mark Lipson works at the U.S. Dept. of Agriculture as the Organic and Sustainable Agriculture Policy Advisor in the Office of the Secretary. From 1995-2010, Mark was the Policy Program Director and Senior Policy Analyst for the Organic Farming Research Foundation. In the mid-1980s, Mark established the statewide office of California Certified Organic Farmers and helped develop the foundational aspects of organic certification systems. He played a key role in the passage and implementation of both the California Organic Foods Foods Act of 1990 and the U.S. Organic Foods Production Act. From 1991-97 he was Chairman of the California Organic Foods Advisory Board. In 1992 he received the Steward of Sustainable Agriculture ("Sustie") Award from the annual Ecological Farming Conference. He served on the USDA Agricultural Biotechnology Advisory Committee in 1999-2002. Mark received his B.A. degree from the University of California, Santa Cruz in 1981 with honors from the Environmental Studies program, in the Planning and Public Policy concentration. Since 1983 Mark has been a member of the Molino Creek Farming Collective, a cooperative multi-family organic farming community near Davenport (Santa Cruz County), California.

Dr. Zhongchi Liu is currently an associate professor at the Department of Cell Biology and Molecular Genetics, University of Maryland, College Park. After graduating from Wuhan University, China with a BS degree in Microbiology, she came to US in 1983 to study under Dr. Victor Ambros at Harvard University. Her PhD work was centered on the identification and characterization of developmental timing genes in *C. elegans.* In 1991, attracted by the emerging model plant *Arabidopsis thaliana* and the elegant genetic study of flower development, she joined the lab of Dr. Elliot Meyerowitz as a postdoctoral fellow at the California Institute of Technology, where she started her career as a plant developmental biologist. Since joining faculty at the University of Maryland in 1995, Dr. Liu has continued her research on the regulatory mechanisms of floral homeotic gene expression in *Arabidopsis thaliana,* with a particular focus on a set of transcriptional co-repressors that control floral whorl-specific expression of *AGAMOUS* and *miR172*. Through her work with floral homeotic genes, she recently ventured into gene silencing and floral fertility control.

The Science of Gene Flow in Agriculture and its Role in Co-existence
September 7-8, 2011
Washington, DC

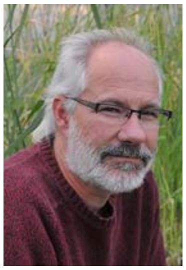
Michael F. Portereiko is a senior scientist and Trait Manager specializing in reproductive biology and gene-expression analysis at Ceres, Inc., where he has worked since 2006. Before coming to work at Ceres, Mike did a three year post-doc with Prof. Gary N. Drews at the University of Utah, where he co-authored several papers on female gametophyte development and the role of *AGAMOUS-like* genes in central cell and endosperm development.

Dr. Carol Mallory-Smith is a Professor of Weed Science at Oregon State University in the Department of Crop and Soil Science. Dr. Mallory-Smith's main areas of research are weed management in agronomic crops, weed biology, herbicide resistance, and gene flow and hybridization between crops and weeds. She teaches courses on Weed Management and Herbicide Science. She has coauthored more than 100 journal articles, 8 book chapters and numerous extension and popular press articles. Mallory-Smith visited Australia and South Korea as an invited expert on gene flow and herbicide resistance. She taught and lectured in Argentina as a Fulbright Scholar in 2009. She is a Fellow of the Western Society of Weed Science and the Weed Science Society of America, served as President and Treasurer of the Weed Science Society of America and Secretary-Treasurer for the International Weed Science Society. Mallory-Smith was named to the Oregon State University College of Agricultural Science Registry of Distinguished Teachers. She received the Alumni Achievement Award from the University of Idaho's College of Agriculture in 2007, the Excellence in Graduate Mentoring Award from Oregon State University and the Western Society of Weed Science Outstanding Weed Scientist in 2009.

Dr. Allison A. Snow is a professor in the Department of Evolution, Ecology, & Organismal Biology at Ohio State University. She received her Ph.D. from the University of Massachusetts and postdoctoral fellowships from NSF and the Smithsonian Institution. Her research combines molecular and ecological approaches to understand how quickly crop genes move into wild populations, and the extent to which novel transgenic traits could benefit weedy and semi-weedy plants. Dr. Snow is the lead author of a 2005 position paper by the Ecological Society of America on environmental effects of genetically engineered organisms. A Fellow of the American Association for the Advancement of Science and the Aldo Leopold Leadership Program, she has served on the editorial boards of Ecology, Ecological Monographs, Evolution, Frontiers in Ecology, and Environmental Biosafety Research. She also served as President of the Botanical Society of America and Treasurer of the International Society for Biosafety Research. She has advised the US National Academy of Sciences, the US Department of Agriculture, the US Agency for International Development, and the World Trade Organization. In 2010, Dr. Snow was invited to advise President Obama's Commission for the Study of Bioethical Issues on the topic of synthetic biology.

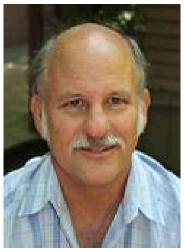
The Science of Gene Flow in Agriculture and its Role in Co-existence

September 7-8, 2011
Washington, DC



Neal Stewart, is a professor of plant sciences and holds the Ivan Racheff Chair of Excellence in plant molecular genetics, and is the co-director of the Tennessee Plant Research Center. He is the project lead for switchgrass transformation for the Oak Ridge National Laboratory BioEnergy Science Center. After taking a PhD at Virginia Tech (1993), he joined Wayne Parrott's lab at the University of Georgia.. After that, Stewart joined the faculty of UNC-Greensboro and was an assistant-, then associate professor from 1995-2002 in biology, after which he assumed the Racheff Chair and at the appointment of professor, and then with tenure in 2006. The Stewart Lab's research spans plant biotechnology, genomics, and ecology. He has been performing agricultural biotechnology and biotechnology risk assessment research since 1994. His research has been supported by various granting agencies including the DOE, USDA, NSF, EPA, NASA and various US military agencies. He is the author of over 200 publications, including 6 books. His work has been cited over 4000 times. He has editorial duties for 7 journals. During his career he has mentored over 80 graduate student, postdoctoral, and technician trainees.

Dr. Steven H. Strauss is a Distinguished Professor of Forest Biotechnology in the Department of Forest Science at Oregon State University, and has a joint appointment in the Molecular and Cellular Biology Program. He is Director of the Tree Biosafety and Genomics Research Cooperative at OSU, a university-public agency-industry consortium formed in 1994. It conducts research on the biosafety and physiology of genetically engineered trees used in plantation forestry and horticulture. Strauss directs the OSU Program for Outreach in Resource Biotechnology, aimed at promoting public understanding, and facilitating science-based public debates, in food and natural resources biotechnology (http://agsci.oregonstate.edu/orb/).



Dr. Larry R. Teuber is a professor in the Department of Plant Sciences, and is affiliated with the Genetics and Horticulture and Agronomy Graduate groups at UC Davis. He received his Ph.D in Genetics and Plant Breeding in 1978 from the University of Minnesota. His research interests include the genetics and biology and production of alfalfa and wild rice. Dr. Teuber has over 35 years of experience in research in many area of alfalfa seed production including the genetics and breeding for floral characters that influence pollination by honey bees, development resistance to lygus bugs and whiteflies, fall dormancy and optimization of planting date relative to seedling development. In recent years, Dr. Teuber's long term interest in pollination has been utilized in a number of studies defining gene flow. He is the former Director of the UC Foundation Seed Program and is currently the Executive Director of the California Crop Improvement Association.

The Science of Gene Flow in Agriculture and its Role in Co-existence

September 7-8, 2011
Washington, DC

Dr. Rene Van Acker is Professor and Associate Dean of the Ontario Agricultural College at the University of Guelph and was previously a professor of weed science and crop management at the University of Manitoba. His research interests include weed biology and ecology, robust cropping systems, multifunctional agriculture and the coexistence of GM and non-GM crops. He conducts field-based research, supervises graduate students and teaches courses at the undergraduate and graduate levels. He has published over 100 peer-reviewed works to-date and has made over 250 other non-peer reviewed contributions. His research work on coexistence of GM and non-GM crops has lead to international collaborations and presentations, and consulting work with governments and organizations in Denmark, Austria, Switzerland, France, Germany, Australia, the US and Canada. Rene grew up on a farm in southwest Ontario. He holds BSc and MSc degrees in crop science and weed management from the University of Guelph and a PhD in crop-weed ecology from the University of Reading in the UK. Rene and his wife Susie have three children and live in Guelph.

Dr. Allen Van Deynze is the Research Director at the Seed Biotechnology Center at University of California, Davis. Dr. Van Deynze received a BSc. and MSc. degree in Plant Science from the University of Manitoba, Canada and a Ph.D. in plant breeding from the University of Guelph, Canada. He did a postdoctorate in molecular genetics at Cornell University in the Department of Plant Breeding and Biometry. He worked as a plant breeder for Calgene/Monsanto and as a senior scientist for Celera AgGen and Paradigm Genetics where he developed and implemented strategies to incorporate biotechnology into breeding programs. As part of the SBC's mission to serve as a liaison between public institutions and the seed industry, he develops, coordinates and conducts research on the application of biotechnologies to seed crops. Dr. Van Deynze generates and disseminates scientific and informational content for the Center's educational and outreach programs. Dr. Van Deynze research interests include identification of molecular markers to help incorporate traits into new varieties, development of new phenotypes.

Gene Flow in Agriculture

Allen Van Deynze, Seed Biotechnology Center, University of California, Davis, CA, 95616

US agriculture thrives on innovation which in turn maintains competitiveness in the global market. Consequently, to capture value, we must enable a diverse set of products and production systems to co-exist and meet the specific market demands. Unlike, many other technology markets, agriculture involves biological systems that only in rare instances allow for 100% purity or 0% “contamination”. This is exasperated by an international commodity handling system with different standards even for the same products. At least three production systems, conventional organic and biotech (transgenic) are used across the US with the latter 2 enjoying double digit growth annually. The factors affecting the level of intermixing of genetic materials through gene flow via pollen, seed and admixtures must be understood to develop practical non-zero thresholds to meet and develop realistic market standards. Furthermore, as novel agricultural products are developed, an understanding of persistence in the environment in wild populations or simply through vegetative propagation such as in perennial grasses being considered for biofuels must be understood prior to release.

Co-existence principles rely on mutual respect among growers and a clear understanding of the biological restrictions of crop production systems. Growers and the seed industry have developed these principles over the last 100 years, adjusting them as new information becomes available and as cropping systems evolve. For example the Association of Official Seed Certifying Agencies (AOSCA) develops and maintains standards for seed purity with 70 member countries. Similarly, the American Seed Trade Association (>800 members) works with the seed industry to ensure practical standards are developed to maintain markets. Under current regulations, USDA/APHIS regulates crops that are potential plant pests to evaluate potential risks of biotech-derived crops to the environment and US agriculture and the National Organic Program has set standards for which materials and processes may be used to certify organic products.

Current mitigation strategies are crop-specific adjusting for the biology of the crop and the environment it is grown. For example, outcrossing rate, pollen type, pollinators, sexual compatibility, presence of related species, seed dispersal, fecundity and dormancy are considered when evaluating risk. Based on these, appropriate distances from compatible plants can be recommended for a level of purity. Crop rotations and specific handling techniques are developed. Seed purity tests (varietal purity, weediness, and inert matter) as well as genetic tests are used to monitor the effectiveness of the co-existence standards (Van Deynze et al., 2008). Recently, conventional and gene-modification technologies have been developed to contain pollen and seed dispersal in plants. Male sterility systems found in many commercial crops have been effectively used in hybrid production, but can be used to control gene flow in crops where seed is not the primary product. There are several reversible male (pollen) sterility systems that are controlled genetically, through the cytoplasm and engineered (Stewart, 2007; Verma and Daniell, 2007). Similarly, there are several systems being developed that can selectively control seed production and production of whole flowers using gene switches. One of the first selective seed systems have been developed by the USDA/ARS (Lee and Natesan, 2006).

The goal of this conference is to have leaders in the field present and discuss the impact of gene flow on agriculture, current knowledge of gene flow and persistence in the environment and current and novel mitigation strategies for gene containment in a conference setting. The information will be synthesized and a whitepaper will be published to capture the information.

References

Lee, D., and E. Natesan. 2006. Evaluating genetic containment strategies for transgenic plants. Trends in Biotechnology 24:109-114.

Stewart, C.N., Jr. 2007. Biofuels and biocontainment. Nat Biotechnol 25:283-4.

Van Deynze, A., S. Fitzpatrick, B. Hammon, M. McCaslin, D.H. Putnam, L. Teuber, and D. Undersander. 2008. Gene flow in alfalfa: biology, mitigation, and potential impact on production, pp. 30 Council for Agricultural and Science Technology. http://www.cast-science.org

Verma, D., and H. Daniell. 2007. Chloroplast vector systems for biotechnology applications. Plant Physiol 145:1129-43.

Economic Impacts of Gene Flow

Nicholas Kalaitzandonakes
University of Missouri, Columbia. 125 Mumford Hall 65211

Introduction

There are instances where genetic purity matters and limiting the gene flow between neighboring crops has economic value. Such instances include the production of planting seeds; of crops with special functional characteristics (e.g. waxy and high amylase corn); of organic crops with specific GMO tolerances; and of non-GMO crops that seek to avoid mandatory GMO labeling or pursue voluntary labeling.

Prevention of gene flow requires re-engineering of the standard production practices used in commodity systems. A variety of interventions may be used, including: geographic and temporal isolation of production, minimum allowable distances between fields, buffers, border rows and other physical barriers (e.g. Bullock and Desquilbet, 2002; Devos et al., 2005; Kalaitzandonakes and Magnier, 2004).

In addition to prevention, remediation is also used when gene flow occurs despite preventive measures. Through repeated testing, remediation strategies seek to identify and isolate non-conforming grain before entering the segregated stream. To be effective, testing must not greatly interfere with the operational efficiency of the segregated supply chain; it must not lead to erroneous results (false positives or false negatives); it must discourage cheating; and it must be cost effective. These factors are taken into account when firms design their strategies and decide where to test, how much to test and what test to use (Wilson and Dahl, 2005; Konduru et al., 2009).

The Economics of Gene Flow Prevention

Changes in farm operations for preventing gene flow as well as testing and remediation involve additional costs. There are both direct and indirect costs (Kalaitzandonakes et al., 2001). Direct costs are payable costs and result from the re-engineering of operations, additional coordination and control (e.g. contracting costs, testing costs, third party certification fees, etc.); and liabilities from product failures (e.g. demurrage costs, costs of dispute resolution, etc.). Indirect costs are non-payable and result from efficiency losses (e.g. underutilization of land due to use of buffer zones) (Kalaitzandonakes et al., 2001; Bullock and Desquilbet, 2002).

The costs of limiting gene flow are not fixed. They can vary significantly across commodities,[1] regions, and over time[2]and a number of factors can influence their relative size. The most significant driver of such costs, however, is the tolerance level set. The costs of limiting gene flow increase as tolerances decrease (Giannakas et al., 2011). The rigor with which prevention strategies are designed and implemented depends mostly on the allowable “margin of error” which is defined by the set tolerances. For segregated crops with low tolerances, strict measures designed to prevent even minute amounts of gene flow must be put in place. Low tolerances also mean additional testing and greater amounts of product failures (Bullock and Desquilbet, 2002; Kalaitzandonakes and Magnier, 2004). Beyond certain levels, as tolerances diminish, segregation costs increase exponentially (Kalaitzandonakes and Magnier, 2004, 2006). Under zero or near zero tolerance, production and trade of the segregated crop will tend to cease (Magnier et al., 2009).

Coexistence

Historically, the segregated crops have sought to isolate themselves from the commodities and prevent gene flow from neighboring crops. As such, they have incurred segregation costs and, in turn, they have commanded premiums and prices higher than commodities. In general, the costs of preventing gene flow and other admixtures have been in line with the premiums segregated

---

[1] For instance, while outcrossing control may require expensive measures in the production of cross-pollinating maize it is a minor issue for self-pollinating soybeans. Similarly, testing costs might be significantly higher in non-GM maize program than in soybean ones due to greater amount of events that one must test for.

[2] Variation in input and commodity prices alone can lead to significant spatial and temporal variations in segregation costs. For instance, large swings in commodity and input prices imply significant changes in the opportunity costs associated with foregone yields and efficiency losses in the production of segregated grains.

received (Kalaitzandonakes and Kaufman, 2006). In this context, the coexistence of segregated and commodity crops has been left to the market to short out.

In recent years, some countries have considered the gene flow between GM and conventional or organic crops an economic externality and have sought to actively regulate it. The cultivation of approved GM crops, for instance, within the EU is regulated by national coexistence policies. Policies for the coexistence of conventional, GM and organic crops have been developed in the EU since the mid-2000. Their stated goal has been to ensure freedom of choice for farmers in their production decisions and for consumers in their purchasing decisions.[3] In practice, European regulations on coexistence have sought to introduce technical and administrative rules on the production of GM crops (e.g. isolation distances, buffer zones, etc.) as well as to define the liability of farmers growing such crops from potential losses of organic and conventional farmers that might occur from accidental admixtures (e.g. EU Commission, 2006, Koch, 2008). Yet even within the EU, national coexistence regulations and their impacts on the use of GM crops are quite diverse. While Spain uses existing regulations to govern the production of GM crops, other countries, such as Bulgaria, have developed coexistence regulations which effectively ban GM crop production. Such rules have prompted some researchers to suggest that coexistence policies in the EU have been discriminatory and punitive against GM crops (e.g. Beckmann *et al*., 2010, Demont et al., 2009, Devos et al., 2008, Ramessar et al., 2010). Other researchers however have proposed that when market externalities caused by GM crops are properly accounted for, coexistence policies in the EU may be justified (e.g. Disquilbet and Bullock, 2010). It remains to be seen whether such regulatory measures succeed in practice to ensure the coexistence they seek.

---

[3] According to the European Coexistence Bureau: *"Coexistence refers to the ability of farmers to choose between the cultivation of genetically modified (GM) and non-GM crops, in compliance with the relevant legislation on labeling rules for GM organisms (GMOs), food and feed and/or purity standards."*

References

Beckmann, V., C. Soregaroli and J. Wesseler, 2010. Ex-Ante Regulation and Ex-Post Liability under Uncertainty and Irreversibility: Governing the Coexistence of GM Crops. Economics: The Open-Access, Open-Assessment E-Journal, Vol. 4, 2010-9.

Desquilbet, M., and D. Bullock "On the proportionality of EU spatial ex ante coexistence regulations: A comment" *Food Policy* 35 (1): 87-90, 2010

Demont, M., K Dillen, W. Daems, C. Sausse, E. Tollens and E. Mathijs "On the proportionality of EU spatial ex ante coexistence regulations" *Food Policy* 34 (6): 508-518, 2009

Devos, Y., M. Demont, and Sandivo. Coexistence in the EU—return of the moratorium on GM crops? *Nature Biotechnology* 26 (11) 2008.

Devos Y., Reheul D, DeSchrijver A (2005) the co-existence between transgenic and non-transgenic maize in the European union: a focus on pollen flow and cross pollination. Environmental Biosafety Research. 4, 71-87

European Commission. "New Case Studies on the Coexistence of GM and non-GM Crops in European Agriculture." Technical Report Series, EUR 22102, Joint Research Center, January 2006. Available at *http://www.jrc.es/home/pages/eur22102enfinal.pdf*

Giannakas, K., N. Kalaitzandonakes, A. Magnier and K. Mattas "Economic Effects of Purity Standards in GMO Labeling Laws" *Journal of Agricultural Industrial Organization*, forthcoming

Kalaitzandonakes, N. and J. Kaufman, (2006) "GM Crops Impact Starch Industry" World Grain, February.

Kalaitzandonakes, N., R. Maltsbarger, and J. Barnes. (2001) Global identity preservation costs in agricultural supply chains, *Canadian Journal of Agricultural Economics* 49(1): 605-15.

Kalaitzandonakes N.G., and A. Magnier. (2004) Bioech labeling standards and compliance costs in seed production. Choices 21:1-6.

Konduru, S., N. Kalaitzandonakes, and A. Magnier (2009) GMO Testing Strategies and Implications for Trade: A Game Theoretic Approach. *Paper presented at the Agricultural & Applied Economics Association, Milwaukee, Wisconsin, July 26-29, 2009*

Koch, B. (ed) Economic loss caused by genetically modified organisms: liability and redress for the adventitious presence of GMOs in non-GMO crops Springer, New York, 2008

Magnier, A., S. Konduru and N. Kalaitzandonakes (2009) Market and Welfare Effects of Trade Disruption from Unapproved Biotech Crops. Paper presented at the Agricultural & Applied Economics Association's 2009 Annual Meeting, Milwaukee, WI, USA, July 26-28, 2009.

Ramessar, K., T. Capell, R. Twyman, P. Christou "Going to ridiculous lengths—European coexistence regulations for GM crops" *Nature Biotechnology* 28, 133–136, 2010.

Wilson, W., and B. Dahl. (2005) "Costs and Risks of Testing and Segregating Genetically Modified Wheat." Review of Agricultural Economics 27(2):212-228, 2005.

.

Maintaining Seed Purity in the Seed Trade Industry

Richard L. Dunkle*
American Seed Trade Association 1701 Duke St. STE 275, Alexandria, VA 22314

Abstract

Seed purity, more appropriately seed product integrity, is maintained within a framework of regulatory (labeling) requirements through the Federal Seed Act, quality standards, seed testing performed by accredited entities, quality management practices, and production under principles and practices of coexistence. Cooperation among seed developers, seed producers, and growers at the local field level, in particular communication among neighboring growers, is necessary for successful coexistence. This presentation, condensed from the American Seed Trade Association paper "Existing U.S. Seed Industry Production Practices That Address Coexistence," describes the role coexistence plays in the maintenance of seed product integrity.

INTRODUCTION

Founded in 1883, the American Seed Trade Association (ASTA) is a trade organization representing more than 700 member companies involved in seed production and distribution, plant breeding and related industries in North America. Its mission is to enhance the development and movement of quality seed worldwide. For that reason, ASTA commissioned the preparation of this paper to describe how the seed industry employs a variety of tools to attend to the different circumstances of coexistence needed to meet market demands. The scope of the paper focuses on seed industry practices within the context of the agricultural value chain. For the purposes of this paper, "coexistence" means the practice of growing, reproducing and handling seed products with different characteristics or intended markets with the goal of successfully achieving intended product integrity and maintaining the economic value of such products.

AGRICULTURAL COEXISTENCE

Coexistence in the seed industry encompasses a set of tools used by the agricultural value chain to manage the complexities of plant biology and seed production systems, overlaid with market driven demands. The movement of pollen through wind and insects; specific aspects of plant reproductive biology; possible presence of weeds in seed production fields; and equipment used to plant, harvest and transport seeds all need to considered. Building upon many generations of experience, coexistence involves agricultural best practices that bring the greatest benefit to all along the agricultural value chain from seed developers to farmers and from retailers to consumers — from field to fork. The coexistence of various production methods is not a new concept to the agricultural community. In agricultural coexistence, there has never been a one-size fits all because each crop brings a new set of market factors and biology to be considered. Farmers are accustomed to producing different crops next to one another, such as waxy, white, dent, pop and sweet corn, hot and sweet peppers, conventional and biotech grains and cotton varieties with different fiber characteristics. Innovation in crop production using modern biotechnology, such as genetic engineering (GE), is only the latest production method to be introduced to agriculture.

Agricultural commodities that provide food, feed, fiber and fuel move through an extremely flexible system that enables product exchange or replacement to maximize efficiencies. Identity preservation is a system that preserves the value of a product throughout its production chain. Farmers use identity preservation to gain premiums when they market specialty crops (such as seed, organic or a particular variety) because they must achieve an agreed upon standard of quality and purity in their harvested product as driven by the needs of the marketplace. Historically, in specialized production sectors, the growers and the rest of the value chain take responsibility for meeting any quality standards for the product's market demand, often through contractual arrangements. Market incentives balance the benefit and the burden at the farm level. Therefore, quality standards that compel coexistence have been market driven and science-based, and reflect practices that are practical, achievable and economically feasible. Ultimately, though, the success of coexistence lies in the communication and cooperation along the value chain.

Coexistence enables the marketplace to work. End users indicate their needs and preferences through the choices they make on the market. These demands drive the response of the marketplace by working back through the agricultural value chain all the way to the seed industry. Premiums and incentives determined by that market are provided to those along the value chain willing to take on the extra work and cost associated with the production of goods which result from identity preservation. Equipped with marketplace information considered with possible incentives, each segment of the agricultural value chain chooses which crops and what production systems they prefer to employ to bring products to all markets.

Quality standards are based upon market expectations and the limits of biological systems. Therefore, thresholds or tolerances are a component of seed quality standards. The marketplace has historically worked out how to address the tolerances and thresholds for commingling or adventitious presence (AP) through these quality standards. The agricultural value chain responds by finding practical levels to work within to meet reasonable market expectations.

SEED PRODUCTION & COEXISTENCE

The U.S. seed industry is committed to bringing quality seed to farmers around the world. There is no substitute for quality seed, and the key to high quality seed is to maintain seed product integrity. Markets send signals to the seed industry about what kind of seed should be produced so that farmers can meet the needs of their customers and the general consumer. With high quality seed and new technology, farmers have more choice than ever before, and the selection of seed is the single most important factor they can control in their operations.

As an identity preserved product, seed must meet a set of quality standards driven by the market and based on science to achieve the varietal purity desired by that market. The concept is very simple – consumer wants are driven back through the agricultural value chain to the seed industry, a crucial starting point in delivering what the customer is demanding. Scientific information, research and new innovations are reviewed to develop the best quality management systems for coexistence. Exchange is encouraged along the value chain to understand what is practical and realistic for the value attached to any identity preserved product.

Seed companies are required to use truthful labeling provisions that allow them to market seed with quality information disclosed in the packaging. The U.S. Federal Seed Act (FSA) regulates the interstate shipment of agricultural and vegetable seeds. FSA requires that seed shipped in interstate commerce be labeled with information that allows seed buyers to make informed choices and that the seed labeling information and advertisements pertaining to the seed must be truthful. National certification standards, coordinated by the Association of Official Seed Certifying Agencies (AOSCA), are primarily found at the state, rather than the federal, level. emstsSeed testing is covered under rules developed by the Association of Official Seed Analysts (AOSA) and/or the International Seed Testing Association (ISTA) which provides a national seed laboratories accreditation system under a quality management system.

Seed sold in the United States normally goes beyond the requirements of the Federal Seed Act. Seed quality standards for variety identity and purity establish a percentage of unintended mixing of other seed varieties, called “off-types,” while maintaining product integrity. Additionally, government policies on Low Level Presence are key to setting realistic parameters for coexistence related to biotechnology, organic and conventional agriculture.

Third parties play an important role in the success of coexistence in seed production. AOSCA promotes and facilitates the movement of seed or plant products in local, national and international markets through the coordinated efforts of official seed certifying agencies acting to evaluate, document and verify that a seed or plant product meets certain accepted quality standards. State crop improvement associations (such as California Crop Improvement Association and Indiana Crop Improvement Association) and members of AOSCA provide local venues for cooperation and communication to develop industry standards based on those of AOSCA for local seed production and house tools with regional relevance useful in implementing a plan for coexistence. The U.S. Department of Agriculture (USDA) is available to provide seed product documentation under the Organisation for Economic Co-operation and Development (OECD) Seed Schemes for certain export markets. In addition, seed companies often times interact with grower associations or organizations that represent a value chain. These relationships encourage dialogue along the value chain about market demands and ways coexistence can best be obtained.

COEXISTENCE TOOLS OF THE SEED INDUSTRY

Seed companies, often through contractual arrangements with seed growers, have procedures in place to maintain a seed variety’s trueness to type and to reduce chances for commingling during each step of the seed production cycle. The industry employs a variety of tools to successfully manage coexistence and general seed production, including seed production practices and quality management systems, contracting, pinning, communication and cooperation.

Production Practices and Quality Management Systems

For all practical purposes, seed is a product of identity preservation and utilizes methods consistent with commercial practices along the supply chain. Coexistence management practices will be dependent on a number of factors including the crop, the region and the growing environment. Seed production requires greater physical purity than commercial agricultural production, and therefore necessitates more physical barriers to reduce the movement of pollen. Physical mixing is another important consideration. Care must be taken to ensure that physical processes such as planting preparation, harvest, seed cleaning/conditioning and subsequent storage do not compromise either seed quality or integrity. These practices may be employed by the seed company directly, by growers contracted to produce the seed on their behalf, or a combination of the two entities. In seed production, a broad range of production practices may be employed or accounted for, and include:

- Intimate knowledge of neighboring crops and the wild plant communities for possible cross-pollination with seed crops;
- Farmer to farmer communication;
- Rotation schemes of crops which reduce pollen exposure from volunteer plants;
- Seed handling so there is no mixing during planting, harvesting and cleaning operations;
- Temporal isolation for pollen release through staged planting times;
- Field/plot selection and identification;
- Isolation distances, largely based on each crop's reproductive system (self- or cross-pollinated);
- Buffer rows;
- Tracking and recordkeeping;
- Pre- and post-harvest cleaning and inspection of planters, harvesters and other equipment;
- Module markers used in harvest;
- Disposal of plant material as appropriate;
- Designated or cleaned transportation vehicles, storage bins, conditioners and ginning facilities as appropriate;
- Continuous visual inspection and rouging of all genetic stocks to remove off-types and weeds;
- Fields inspections multiple times, possibly by third parties; and
- Post-harvest risk mitigation, such as not harvesting outside rows, if cross-pollination has occurred after planting.

A quality management system takes production practices used in coexistence and puts them into a structure. Quality standards are based upon market expectations and the limits of biological systems. Therefore, thresholds or tolerances are a component of seed quality standards. Maintaining a seed variety's trueness to type is critical for market acceptance. Isolation and containment are used to strive for the greatest purity. A percentage of off-types are part of any practical quality standard at the different levels of seed production – breeder materials, seed stocks and commercial seed. The standard is tighter in the early stages of seed production (i.e. breeding). As seed production is ramped up and broadened, purity expectations are less stringent, but always mindful of the product integrity needed for commercial seed sales. AOSCA seed standards may serve as the basis for any seed quality management system. The baseline standards of AOSCA have been in place for many years. They are based on pollen flow research conducted by universities with the best science at the time. AOSCA makes available to its members the *Seed Certification Handbook* (2009 is the latest edition). Although this handbook is available only to members, the certification standards are generally made public through the state crop improvement associations.

The seed industry uses the AOSCA standards as a minimum, and seed companies may increase their internal quality management requirements depending on the market or type of standard they are trying to meet. General measures are developed by the company into a quality management system for the variety of seed being produced and include identity preservation programs, grower training and inspections. These in-house procedures either meet or go beyond this base level depending on what the market dictates.

The U.S. seed industry, through ASTA, has developed the *Guide to Seed Quality Management Practices* to offer general guidance for the development and production of seed products intended for use in food, feed, fiber or fuel and for the maintenance of product integrity and purity of both biotechnology-derived seed and non-biotechnology seed. The guide covers the plant product life cycle from the point of incorporation of a trait into a breeding program through commercial seed production and sale. The guide is intended to serve as a reference document for companies developing individual quality management practices and operating procedures. In determining how best to use this guide, companies should consider the needs of the marketplace and customer demands so that the appropriate practices and procedures become a normal part of the business process. The *Guide to Seed Quality Management Practices* is maintained in a dynamic and interactive format on the ASTA website and can be found directly through www.amseed.org/seedquality. This interactive structure allows companies to easily refer to the entire guide or specific sections.

Quality Management Systems, such as ISO 9001, provide structure and rigor to business practices by way of managing key process variables, thereby establishing routine and consistent output from their processes. In addition, these systems facilitate coexistence among growers, meeting customer expectations and providing mechanisms for continual improvement of the quality management system. Tracking, recordkeeping, testing and other measures with appropriate oversight management systems are essential parts of product development and commercial life cycle for purposes of quality control and seed purity. An underlying consideration throughout any quality management system is the importance of communication with neighboring seed growers, farms and residences as appropriate. Since maintaining a seed variety's trueness to type is critical for market acceptance and use, robust quality management practices are needed for both biotechnology or non-biotechnology derived crops.

Contracting

Contractual arrangements with seed growers are an integral step in maintaining seed product integrity. Although the seed company contracts with the grower, the grower is normally responsible for the resolution of problems with neighbors. Contract terms with the grower may include:

- Specific instructions to reduce risk of off-types such as isolation distances, pollen rows and conditions (types of crops planted in previous years);
- Possible allowance for some modifications and use of post-harvest practices to reach standard requirements in the final seed lot;

- Contract requirements which include a stipulation of the purity standard the seed lot must meet;
- Previsions for the company to check the isolation distances such as buffers, border rows and weather effects; and
- Purity testing through either visual inspection or through genetic analysis, as well as post-harvest grow outs and bin inspections.

Corn seed production in the Midwest is a good example of the successful use of contracts to manage coexistence. In general, seed corn growers are required through contractual obligations to meet minimum isolation distances requirements and other production practices. They are then responsible for the cooperation and communication among their neighbors to ensure any potential problems are prevented or minimized. If commingling should arise after planting through pollen drift, which jeopardizes the product integrity of the seed, there are measures a grower can take to deliver the final seed product within the contracted specifications. These corrective measures, such as not harvesting the outside rows for seed, would be part of an overall quality management system and outlined in the procedures and guidance given by a seed company to a contracted grower. Some companies choose to have a very close relationship with the grower and provide resources, support and machinery and have ongoing contact. Others contract with the grower, but are not in continuous, direct involvement with the actual production of the seed.

Specialty corn (white, waxy, pop, sweet) is a small portion of the total corn seed production, less than 10 percent. The quality standards are more rigorous with greater purity requirements (i.e. a lower level of off-types is permitted) and therefore require greater isolation distances. Because of these more rigorous quality standards, the contract grower for seed production of specialty corn will normally receive a greater premium which balances out the benefits and the burden on the farm through this market incentive.

Pinning Maps

In some geographic areas, the management of seed crop isolation distances is accomplished through pinning maps. The concept of “pinning” has been a coexistence tool of the seed industry for years. Every year there is a meeting, or “pinning party,” of those involved in seed production (at the state or a more regional level) to agree upon the geographic areas dedicated to different types of seed production and any other specialty crop production. A third party (a state crop improvement association or university Extension office) manages the map and log for the pertinent information. Pinning is not a legal arrangement but more of a “gentleman’s agreement” between companies, and generally, disputes are resolved without litigation.

In most states, a large wall-mounted map is housed at county Extension offices or at the state crop improvement association and is made available to growers and seed company representatives. Many different seed companies in a region come together and foster strong working relationships to create and maintain isolation for seed production. Companies agree to seed quality standards and minimum distances based on buyer requirements. Isolation distances are agreed to by the seed companies based on topography, varieties, volunteers, weeds, insects and weather patterns.

Seed companies bear the responsibility for pinning. As a company signs contractual arrangements with seed growers, the location of each farmer's field is put on a map with a physical color-coded pin to represent the spatial and temporal isolation needed for its production. The pin indicates the exact site and date of planting a company will use for a particular crop and variety. Today, advances in software design, such as that by the California Crop Improvement Association, offer a secure Internet-based map pinning platform.

The parameters underlying the pinning maps can depend upon a number of factors. Isolation distances vary by density of insect pollinators, presence or absence of physical barriers, direction and intensity of prevailing winds and acreage planted to seed crops. Numbers of insect pollinators can increase based on ambient temperature, humidity and the agro-ecosystem. Physical barriers such as terrain, vegetation and buildings can significantly decrease the flow of pollen between crops and decrease necessary isolation distance. Prevailing winds and the physical size of the seed can also be important determinants in the amount of pollen present in areas adjacent to seed production plots. Cross-pollinated wind-pollinated species such as beets, Swiss chard and spinach require greater isolation than most other insect-pollinated cross-pollinators. Isolation from GE crops is important to organic producers and in other markets with low or no tolerance for biotechnology material.

Communication & Cooperation

The integrity of the seed industry and the products which they deliver is achieved collectively. Cooperation among seed developers, seed producers and growers at the local field level, particularly communication among neighboring growers, are necessary to sustain coexistence. Early communication is necessary so placement and contracting of fields can be accomplished in time for the seed production to begin. Third parties play an important role in this communication.

The spirit of cooperation creates the opportunity for ongoing dialogue when no crisis exists, building more trust among the parties and in the process itself. Growers are able to dissolve problems when they arise because of the cooperation they have built through the years. They may decide to change a field site, increase the isolation distance or select a planting date which decreases the risk of pollen flow.

FUTURE

The seed industry responds to the marketplace to deliver high quality seed in a multitude of crops utilizing a variety of production systems. As consumer preferences change, new innovation, technology and science are applied to the existing tools utilized by the U.S. seed industry to ensure the success of coexistence. New tools and the evolution of proven techniques, such as pinning or contracting, allow seed companies to best respond to what the market signals, providing appropriate financial incentives to those who take on the risk and work associated with the production of this specialty crop, seed. The dynamic nature of agriculture is apparent in today's seed industry, poised to supply high quality seed desired by all customers of food, feed, fiber and fuel.

RESOURCES

American Seed Trade Association Guide to Seed Quality Management Practices. General guidance for the development and production of seed products for the maintenance of product integrity and purity of both biotechnology derived seed and non-biotechnology seed, covering the stages of plant product life cycle from the point of incorporation of a trait into a breeding program through commercial seed production and sale. More information can be found at www.amseed.org/news_seedquality.asp

Association of Official Seed Certifying Agencies. The Association of Official Seed Certifying Agencies (AOSCA) sets out the minimum standards for seed purity and seed identity. It also recommends minimum standards for seed quality for the different classes of certified seed. More information can be found at www.aosca.org.

California Crop Improvement Association. California Crop Improvement Association (CCIA), a non-profit corporation, is officially recognized as the seed certifying agency under the California Seed Law. The mission of CCIA is to provide services and support research that promotes the improvement, production, distribution and use of superior quality seeds and other agricultural products. More information can be found at http://ccia.ucdavis.edu/.

Federal Seed Act. Under the Federal Seed Act (FSA), the United States Department of Agriculture (USDA) Agriculture Marketing Service (AMS) regulates the interstate shipment of agricultural and vegetable seeds. The FSA includes several definitions of seeds by class, Breeder, Foundation, and Commercial, as well as labeling requirements. More information can be found at www.ams.usda.gov/lsg/seed/geninfo.htm.

International Seed Testing Association. The International Seed Testing Association (ISTA) develops and publishes standard procedures for sampling and testing of seeds. ISTA also runs an accreditation program. More information can be found at www.seedtest.org.

National Alfalfa & Forage Alliance. The National Alfalfa & Forage Alliance is an alliance between the growers, genetic suppliers and university segments of the alfalfa and alfalfa seed industry. More information can be found at www.alfalfa.org.

Organization for Economic Cooperation and Development Seed Schemes. The Organization for Economic Cooperation and Development Schemes for the Varietal Certification of Seed Moving in International Trade (OECD Seed Schemes) promotes the use of agriculture seed of consistently high quality. Certified seeds are produced - and officially controlled - according to common harmonized procedures in 58 participating countries. More information can be found at www.oecd.org/agr/seed.

Implications of Gene Flow for Organic Agriculture

Mark Lipson[1]

Abstract: The unintended and undesirable introgression of crop traits (i.e. "gene flow") into crops, production systems, and economic value chains is a concern of U.S. policy makers. This overview offers a policy-analysis framework for empirical investigations of the impacts on U.S. organic agriculture from gene flow. A classification is offered to distinguish impacts as regulatory, commercial or agroecological. Examples of each category are given.

Introduction

Recent federal court decisions have directed USDA to more closely examine the socio-economic consequences of gene flow, i.e., the unintended transmission of transgenic crop traits to agricultural systems not intended to contain such traits. These court actions coincide with increased quantity and diversity of new transgenic crop varieties which are likely to enter commerce following deregulation under USDA's authority. At the same time, sensitivities to the presence of transgenic crop traits are increasing in a number of markets. USDA is seeking to minimize these conflicts and associated litigation. Thus there is a need for USDA policy makers and regulators to gain a better understanding of the mechanisms of gene flow and its consequences.

One tool in this effort is the USDA Biotechnology Risk Assessment Research Grants (BRAG) program. BRAG's general purpose is, "to support the generation of new information that will assist Federal regulatory agencies in making science-based decisions about the effects of introducing into the environment genetically engineered organisms (GE)."[2] While the BRAG program is specifically concerned with the biological and environmental risks of transgenic crops, the studies conducted through this program are applicable (and necessary) to the wider task of minimizing conflicts arising from transgenic gene flow.

---

[1] United States Department of Agriculture, Office of the Secretary, Marketing and Regulatory Programs, Organic and Sustainable Agriculture Policy Advisor. 1400 Independence Ave., SW, Washington DC 20250.

[2] United States Department of Agriculture, *Biotechnology Risk Assessment Research Grants Program, 2011 Request for Applications*, p. 5. Accessed August 25, 2011 at <http://www.nifa.usda.gov/funding/rfas/pdfs/11_brag.pdf>.

A brief overview of GE gene flow impacts for the organic sector[3] is offered here, and a typology for differentiating these impacts is suggested. For purposes of policy analysis, the impacts of gene flow can be categorized as regulatory, commercial and agroecological. A single mechanism of gene flow (e.g., cross-pollination between nearby fields) has distinct implications at each of these levels.

Regulatory Implications: Prevention Required

Regulatory requirements for organic producers and handlers are derived from the Organic Foods Production Act of 1990 (OFPA) and the implementing regulations (7CFR Part 205 *et seq*). OFPA predated significant adoption of transgenic crops and did not explicitly account for the issue of unintended GE presence in organic products. The Final Rule implementing the Act was issued in 2000 when the issue was recognized but not yet widely discussed nor studied in any depth. The *intentional* use of GE was indeed widely and energetically debated during the rulemaking process from 1997-2000. The proposed organic regulation received a record-breaking number of public comments and the vast majority of these opposed the inclusion of GE as an acceptable component of organic production. Therefore the final rule established a category of "excluded methods[4]" which included genetic engineering[5].

The Preamble to the Final Rule stated that:

> As long as an organic operation has not used excluded methods and takes reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan, the unintentional presence of the products of excluded methods should not affect the status of the organic operation or its organic products[6].

Therefore the regulatory impact of unintended GE presence is not necessarily to effect a disqualification from organic status or a decertification of an organic operation. The critical caveat is the, "…reasonable steps to avoid contact with the products of excluded methods." The National Organic Program (NOP)

---

[3] It is important to note that market sensitivity to GE traits is not limited to "Organically Produced" agriculture and other claims which eschew transgenic methods *per se.* Market sensitivities also include large-scale global markets with divergent approvals of specific transgenic traits. I hope that this presentation on the organic sector will also be helpful to the discussion of other segments.

[4] See discussion in the Rule's preamble at 65 Fed. Reg. 80547-80549 (December 21, 2000), and rule language at 7CFR 205.105.

[5] The definition of "genetic engineering" for purposes of the Organic Regulation is at 7CFR 205.2.

[6] 65 Fed. Reg. 80556 (Dec. 21, 2000).

recently updated its guidance on the issue of “reasonable steps to avoid” GE materials in a July 22, 2011 Policy Memorandum.[7]

For purposes of this discussion, the main example of avoiding contact with GE materials is the prevention of cross-pollination from GE crops. In this case, organic producers are *required* by the regulation to utilize physical barriers (e.g., buffer strips) and management practices (e.g, planting times staggered from neighbors) to prevent cross-pollination. In some cases this can also include seed testing, e.g., if contamination of foundation seed or non-GE seedstocks is known to exist. If preventive measures are not taken, a grower may be found in violation of the regulation. If measures are taken and they are ineffective, further measures may be required or the grower may not be able to produce that particular crop.

The costs of these preventive practices are usually borne entirely by organic producers. Published data on these costs is not yet available. The efficacy of these preventive practices is an important aspect of studies conducted under the BRAG program. Likewise, improving the technical and logistical means to maintain high-quality, GE-free germplasm and seed supplies are priority needs for the organic sector. The importance of real-world field measurements to investigate preventive practices and seed purity methods under varied conditions and locations cannot be over-emphasized.

Commercial Implications: Loss of Sales, Markets, and Consumer Confidence

Despite the process-based nature of the organic regulations and their wording, which allows for regulatory acceptance of some unintended presence, the marketplace can and does impose much more stringent requirements upon organic growers and handlers. In response to perceived demand as well as foreign market requirements, a number of organic food manufacturers are testing and sometimes rejecting loads of organic commodities (corn, soybeans, cotton and canola) due to GE presence.[8]

---

[7] USDA National Organic Program, Policy Memorandum 11-13, found at http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5090396 on August 25, 2011.

[8] Greene, C. and Smith, K. “Can Genetically Engineered and Organic Crops Coexist?” Choices Magazine, 25(2), accessed at http://www.choicesmagazine.org/magazine/article.php?article=131, August, 25, 2011.

Market requirements for the absence of GE materials have caused organic producers and handlers to lose specific sales and price premiums[9]. Repeated inability to meet evolving market requirements may affect access to entire market segments. Ultimately, consumer expectations about the absence of GE materials may become sufficiently at odds with the NOP regulatory limitations, such that confidence in the USDA label could be diminished with the attendant economic losses.

Agronomic and Agroecological Implications: System Degradation

Systemic harm to organic cropping systems may be another category of gene-flow impacts. Although this has been discussed by organic producers, there is little empirical literature on this topic.

One example of systemic impacts is the collapse of organic canola production in the U.S. and Canada due to contamination of canola foundation seed by herbicide-tolerant GE traits[10]. As described in Congressional testimony by North Dakota farmer Dr. Fred Kirschenmann, the implications go beyond the economic loss to a more fundamental level of agroecosystem management:

> Successful organic farming depends in large part on putting together a mix of crops in a complex crop rotation plan…The loss of any crop in this complex crop rotation system presents significant management problems…Canola was a critical crop for us…[11]

Thus the uncontrolled gene flow from GE canola varieties may have seriously undermined the adoption of organic grain systems in the upper Midwest by taking out a critical plank in the agroecological system. Investigation and analysis of such potential long-term effects is needed.

Conclusion

This brief overview suggests the need for a comprehensive and detailed analysis of the implications of gene flow for the U.S. organic sector. Many of these implications pertain to other non-transgenic systems. Policy makers need empirical data on the effects of gene flow for each of the three categories suggested.

---

[9] *Ibid.*

[10] Smyth, S. et al, 2002. “Liabilities and economics of transgenic crops,” Nature Biotechnology, Vol. 20, June 2002, 537-541.

[11] Kirschenmann, F., 2010. Cultivating and Ecological Conscience. University Press of Kentucky, Lexington KY. 111-112.

Importance of Gene Flow to Germplasm Conservation and Development

Stephanie L. Greene*
USDA, ARS National Temperate Forage Legume Genetic Resources Unit, 24106 North Bunn Road, Prosser, WA 99350.

Abstract

Gene flow is an evolutionary process that plays an important role broadening and maintaining genetic diversity in our crop species, especially those that are cross pollinated. This paper defines gene flow and discusses its importance in contributing to the genetic diversity we see today in crop germplasm. It also discusses the need and challenges to minimize gene flow so that we can adequately conserve germplasm for future development.

Introduction

Depending on the circumstances, water flowing downhill can provide irrigation to fields or flood villages. Gene flow can also be positive or negative depending on the circumstances. For millennium, farmers have been relying on gene flow between crops and wild and weedy relatives to introduce useful variation that results in better adaptation and higher yields. Plant breeders today directly harness gene flow by crossing desirable parents and selecting the best progeny to develop modern varieties. Gene flow, occurring naturally or artificially between populations of the same species has resulted in crop gene pools that provide a reservoir of genetic diversity we rely on to meet ongoing production challenges. This reservoir of genetic diversity is often referred to as germplasm. To ensure adequate diversity for future use, crop germplasm needs to be conserved. An important objective in conserving germplasm is maintaining the genetic integrity of original samples. To implement this objective it's important to minimize inadvertent gene flow. The purpose of this paper is two-fold. 1) We'll define gene flow and discuss its importance in contributing to the genetic diversity we see today in crop germplasm; 2) we'll discuss the need and challenges to minimize gene flow so that we can adequately conserve germplasm for future development.

The Process of Gene Flow

In the natural world the ebb and flow of genes between individuals of a species is an important process that endlessly reshuffles genetic diversity. Slatkin (1987) points out that the hallmark of gene flow is the introgression of incoming alleles into the progeny of the recipient population. Both pollen and seed can be sources of gene flow. Fresh variation in introgressed progeny is molded by the forces of natural selection, giving rise to the differences we see within species and supporting the evolutionary forces that lead to speciation. Arriola (2005) points out the factors involved that result in the successful inclusion of incoming alleles into subsequent generations. Hybridization needs to occur among individuals between populations. This means populations need to overlap in terms of geography and flowering times; they need to be sexually compatible and pollination vectors need to be the same. Once hybridization occurs, hybrid progeny need to persist in the recipient populations. This means introduced alleles cannot have a negative impact on fitness. If introduced traits convey an adaptive advantage, not only will the alleles persist, but subsequent generations will reflect an increased frequency of introgressed alleles. The literature abounds with examples of gene flow between crops and their wild and weedy relatives (For a review, see Ellstrand 2003a). There are also reports of gene flow between genetically engineered (GE) varieties, and their crop wild relatives and wide ranging discussions on the implications of such gene flow (Quist and Chapela 2001; Gepts and Papa 2003). Although less extensively studied, concern about gene flow between transgenic crops and conventional crop varieties is also a concern (Ellstrand 2003b).

Conserving Germplasm by Minimizing Gene Flow through Pollen and Seed

Genebanks have long been aware of the importance of maintaining the genetic integrity of the individual accessions housed in germplasm collections. Throughout the process of collecting, maintaining and distributing germplasm, ample opportunities arise that influence the original genetic makeup of accessions (Visser and Ramanatha Rao 2005). Gene flow is particularly a concern for crop species that are pollinated through outcrossing, either with insect vectors or wind. Many self pollinated species also have a small rate of outcrossing, which needs to be addressed. Germplasm management strategies are used during regeneration to isolated individual accessions to minimize the chance encounter of alleles from other accessions or compatible crop species grown in the area. As GE crops come on line, discussions on the importance of maintaining germplasm collections free of GE traits has emerged. The question remains if the traditional strategies of controlling pollination are sufficient. Is testing required after regeneration to ensure non-detect levels of GE traits? Additionally, what role does seed-mediated gene flow play? Should we begin to routinely test incoming seed lots to detect the adventitious presence of GE traits? Considering budget constraints, can testing methods be developed that are cost effective? Can thresholds be established that satisfy both collection users who are concerned about GE presence and collection managers who are charged with conserving and distributing germplasm around the world, but have finite resources? These issues need to be explored in coming years as more GE crops come on line.

References

Arriola P.E. 2005. Gene flow, hybridization and introgression: definitions and Explanations. In: de Vicente MC (ed.). Gene flow and germplasm management. Topical Reviews in Agricultural Biodiversity. International Plant Genetic Resources Institute, Rome, Italy.

Ellstrand NC. 2003a. Dangerous liaisons? When cultivated plants mate with their wild relatives. Johns Hopkins University Press, Baltimore, Maryland, USA.

Ellstrand NC. 2003b. Current knowledge of gene flow in plants: implications for transgene flow. Phil. Trans. R. Soc. Lond B 358:1163-1170

Gepts P and Papa R. 2003. Possible effects of (trans)gene flow from crops on the genetic diversityfrom landraces and wild relatives. Environmental Biosafety Resources 2:89–103.

Quist D and Chapela IH. 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. Nature 414:541–543.

Slatkin M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Visser, B. and Ramanatha Rao, V. 2005. Gene flow and the management of ex situ collections. In: de Vicente MC (ed.). Gene flow and germplasm management. Topical Reviews in Agricultural Biodiversity. International Plant Genetic Resources Institute, Rome, Italy.

The potential impact of gene flow mitigation on agriculture

Kent J Bradford
Seed Biotechnology Center, 1 Shields Ave, University of California, Davis, CA 95616

Abstract

Gene flow via pollen or seed dispersal occurs routinely in agriculture and generally has little economic impact. It can have detrimental effects on particular commodities, particularly for seed production, and in the case of genetically engineered (GE) varieties, for organic growers or exporters to GE-sensitive markets. Recently, both court cases and regulatory rulings have sought to define and prevent or mitigate economic injury that may occur due to gene flow. The conditions being proposed for such mitigation are analyzed here with respect to the effects that they would have on those who would be required to implement those conditions. Logical inferences drawn from principles established or sought with respect to the mitigation of gene flow could also have significant impacts for those who are seeking to establish those principles. Both intended and unintended consequences of gene flow mitigation itself could have major impacts on all segments of agriculture. Indeed, the impacts of proposed mitigation strategies could vastly exceed the impacts due to gene flow.

Introduction

Gene flow in the broad sense is a fact of life in agriculture and always has been. While there has been a strong tendency during crop domestication toward self-pollination, out-crossing has seldom been reduced to zero, and many crops remain partially or fully out-crossing. In the past, this continual out-crossing helped to introduce and maintain diversity in crops, but also prevented the development and maintenance of superior varieties, as they would gradually lose their uniformity and unique characteristics over time. In the early days of public breeding programs, varieties were released and propagated by farmers saving their own seed, which over time resulted in genetic drift and loss of varietal characteristics. In response to this, seed certification programs were established in the 1930's and 1940's that established guidelines, particularly pedigrees, isolation zones and field inspections, that maintained genetic identity and enabled farmers to buy seeds that remained true to the original variety type. The development of hybrid varieties, which require controlled pollination between two specific inbred parents, placed further emphasis on controlling pollination to produce even higher quality seeds and varieties. Thus, particularly in the seed industry, there is a long history of managing and controlling pollination to limit or encourage gene flow, depending upon the desires of the breeder or seed producer. In both the seed industry and in subsequent commodity production, the minimum thresholds are generally set at levels that are above zero, but that do not compromise the product for its intended use. Thus, a certain fraction of inbred seed may be allowed in hybrids, and commodities can contain low levels of even quite noxious compounds or materials, such as mycotoxins or insect parts, if they are below thresholds that are established based on experience to be innocuous in the final product.

In regulating the products of modern biotechnology using recombinant DNA methods, this long-established framework was no longer followed. Distinct regulatory requirements were established that mandated no movement or gene flow outside of specific boundaries, and countries established regulatory agencies that could approve or prohibit specific genetically engineered (GE) or biotech products. Rather than thresholds based upon actual intended use, very low (or zero) thresholds were established in many cases, and extremely sensitive tests are available to detect transgenic events. Consequently, even extremely low levels of transgene presence can cause rejection of shipments, fines, law suits and economic losses. Certain production systems, notably organic, ban the intentional use of biotech crops, and though not required by the rules of the U.S. National Organic Program (NOP), seek to prevent all presence of GE products in organic commodities. Exporters of seeds and commodities to countries or markets that reject GE products also face economic losses if transgenic materials are detected in their shipments. Thus, much attention has been focused on the negative consequences of gene flow on certain segments of agriculture, particularly exporters and organic producers (Stokstad, 2011). Both must satisfy customers who prefer not to have genes introduced using recombinant DNA methods in their seeds or products. Those are real concerns, and they deserve to be heard and addressed.

However, conspicuously missing from the discussion to date about gene flow and co-existence of different segments of agriculture are the potential impacts that the steps that might be taken to reduce gene flow could have on agriculture. Discussion has focused on the claims of injury by the affected segments of the agricultural industry, how to address them, how to limit gene flow or mixing, how to compensate those affected when it occurs, etc. Even apart from considering offsetting benefits overall from the biotech crops that have been introduced, the consequences of gene flow and the overall costs and benefits of the mechanisms that have been proposed to mitigate gene flow also need to be addressed. Much of the rest of this workshop will be about the biology of gene flow and how to control and mitigate it. I propose to address a more near-term issue of regulatory and legal issues related to gene flow and co-existence and examine the underlying assumptions and logical implications of the current developments on this topic.

Background

Biotech crops were introduced on a large scale in the US 1996. Since that time, biotech varieties have been adopted by over 85% of growers of those crops for which they are available, not only in the U.S., but also in Argentina, Brazil, China, India, South Africa, and Australia. It is impossible to imagine that this widespread adoption would have occurred without real production and economic benefits to the farmers, and there is extensive evidence that farmers are receiving significant economic benefits from these crops (Brookes and Barfoot, 2008, 2009). The NOP was established in 2000 and certified organic production has increased rapidly since that time. However, organic production remains less than 0.7% of cropped area and approximately 1.1% of total farmgate agricultural value in the U.S. (Economic Research Service, 2008). The management practices that are allowed in certified organic production are specified by the NOP, and do not include the intentional use of biotech varieties. The NOP is clear that the mere presence of GE material is not a cause for loss of organic certification, and there are no thresholds for the presence of GE material in organic products. Nonetheless, most consumers of organic products assume that they are free of GE material, and “GMO-free” is an important marketing claim for organic foods.

This has created a situation in which the relatively small organic production area is surrounded by conventional agriculture, which has broadly adopted biotech varieties, creating the opportunity for out-crossing, mechanical mixtures, and other means that can result in the presence of GM materials in organic products. The same is true for many pesticides used in conventional agriculture that are banned in organic production. In that case, specific thresholds have been established to recognize the desire of organic producers to avoid them, but also in response to the pragmatic problem of being unable to completely do so in an environment where the vast majority of agriculture is using those pest control methods. In the case of GE products, however, no thresholds have been established and some assert that absolute freedom from GE materials is required. With this background, I will explore here not the consequences for the organic sector, but rather the consequences for the conventional sector of agriculture of the measures being proposed to mitigate gene flow to organic (or other non-GE) crops. As much of the current discussion is triggered either by regulatory rulings by USDA-APHIS or law suits filed to challenge those rulings, the rulings and the suits will be the sources for much of what follows.

Fundamental rights

In December 2010, the USDA issued its final Environmental Impact Statement (EIS) and ruling that de-regulated glyphosate-tolerant (GT) alfalfa (for the second time) (USDA-APHIS, 2010). The EIS was done following a suit filed by the Center for Food Safety (CFS) and others challenging the agency's 2005 deregulation of the same varieties. In March of 2011, the CFS and others filed suit challenging the EIS and ruling again (Center for Food Safety v. Vilsack, 2011). In that suit, the plaintiffs claim that the release of GT alfalfa will make it impossible to source GE-free alfalfa seeds and that "The [USDA's] decision threatens the fundamental right of conventional and organic farmers to sow the crop of their choice." To address that issue, the USDA proposed a third alternative (in addition to either fully or not deregulating GT alfalfa) in which certain restrictions would be placed on where GT alfalfa seed (and in some cases hay) could be produced. These proposed restrictions included the following:

- In certain states (those producing more than 1% of alfalfa seed, all in the western US), GT alfalfa for forage could not be planted in counties where alfalfa seed was grown, and
- 5 miles of isolation would be required around GT alfalfa seed fields.

In California in particular, both alfalfa forage and seed are important crops, and large counties in the San Joaquin Valley (including the top four agricultural counties in the U.S. with over $12 billion in agricultural value) would have been forced to decide whether they would grow either seed or forage. Dr. Dan Putnam of the University of California, Davis, estimated that implementation of the proposal would have restricted the crop choices for 57% of the alfalfa acres in the state. The "fundamental right" claimed in the law suit to sow a crop of your choice apparently applies only to the growers of organic alfalfa and would not extend to farmers who choose to plant GT alfalfa. Fortunately, this alternative was not adopted in the final deregulation, and the alfalfa seed production industry has voluntarily adopted a 5-mile isolation buffer around GT alfalfa seed fields, almost double the distance that data indicate would be required to reduce pollen flow to insignificant levels.

A similar situation is currently occurring with GT sugar beets. A judicial decision stemming from a law suit has required that the USDA conduct an EIS in connection with its prior deregulation of GT sugar beets (Center for Food Safety v. Vilsack, 2010). While the EIS is being conducted, the sugar beet industry is operating under USDA-issued permits that impose strict requirements for management, auditing and reporting. Returning again to the language from the March 2011 alfalfa suit: "Other onerous measures carried out in an attempt to prevent contamination will similarly burden Plaintiffs Family Farmers." Growers of GT sugar beets are also Family Farmers, and they are being burdened by the "onerous" conditions imposed by the current permit while an EIS is being conducted. The intent of the suit was to "enjoin any planting, sale or dissemination of Roundup Ready sugar beets", in other words to deny users of GT sugar beets their "fundament right to sow the crop of their choice." If such measures are onerous to or violate fundamental rights of one group, why are they acceptable to be imposed on another group, particularly when the latter represent 95% of that industry, and there is no organic sugar beet production in the US to be protected?

In fact, the basis of the GT sugar beet case was not protection of sugar beets *per se*, but rather the possibility that pollen from GT sugar beet seed production would cross-pollinate organic chard and red beet seed crops in the same area. Sugar beet, red beet and chard are the same species (*Beta vulgaris*) and can cross pollinate (McGinnis et al., 2010). Virtually all of the US production of sugar beet seed occurs in the Willamette Valley of Oregon, which has an ideal climate for producing seed of this biennial crop. It is therefore also an ideal climate for chard seed production. However, knowing the potential for cross pollination, one has to ask whether it is sensible to establish a chard seed production operation in the nation's primary sugar beet seed production location. Seed companies often move to different locations to assure isolation, and cooperate together to coordinate locations and planting dates to achieve required isolation. However, in this situation organic growers are being actively encouraged to produce and save their own seed of these crops related to sugar beet (Organic Seed Alliance, 2010).

What could be the consequences of this situation? Prevention of gene flow through pollination is basically a matter of distance. Under the USDA permit system currently in place, a four-mile isolation distance was mandated between GT sugar beet seed fields and all other *Beta* crops. It would take only 10-12 chard or red beet growers to eliminate sugar beet seed production in the lower Willamette Valley. With the 10-mile radius requested by the plaintiff in the lawsuit, it would take only two. So an industry growing 1.1 million acres of sugar beets and worth $1.5 billion in farm gate value alone could be unable to produce seed of its desired varieties in an established seed production area in order to protect a plaintiff who claimed potential (not actual) lost value of $15,000 in chard seed. We seem to have lost any sense of proportion here, which is an essential underpinning for co-existence.

Liability and compensation funds

Some groups have called for the establishment of compensation funds that would pay not only for any losses that might actually occur in the market due to low level presence of GE material, but also costs for "Segregation and commingling prevention plans, including on-farm and post-harvest and all related supply-chain integrity costs," and "On-going buffer zone control, including production acreage losses and on-going maintenance required to secure or maintain access to contamination-sensitive markets." (National Organic Coalition, 2011). In other words, it is asserted that the GE sector of agriculture should pay for all the costs to enable the non-GE sector to access its higher value markets with standards that require additional buffers, identity preservation plans, etc. In contrast, it is understood in the seed industry (and is also an established legal principle) that the costs of producing and achieving higher quality products are borne by those who will benefit from the higher value. Seed companies cannot simply announce that they are going to be producing hybrid seed, and therefore everyone within a 3-mile radius of their intended location is banned from growing that crop, and in addition, require those neighbors to pay for all of the additional measures it will take the seed company to actually produce the hybrid seed crop to meet the market standard.

It should also be noted that the NOP is clear that inadvertent commingling with biotech products does not in itself entail the loss of organic certification, and the NOP is silent on any thresholds for GE presence (NOP Final Rule, 2000). Thus, inadvertent low level presence of GE material does not prevent either certification or marketing of organic products. Nonetheless, the National Organic Coalition has stated in its guidelines for a compensation fund that "GMO contamination would be based on lowest detectable levels of transgenes and would ratchet down, as improved technology is available." In other words, there would be no threshold, and in fact efforts would be made to detect at as low a level as possible. This is proposed even though surveys of organic growers and marketers have found very few cases where organic products have been rejected because of GE presence, and 92% of respondents in one survey "reported incurring no direct economic costs" related to GE crops and only 1% reported any loss of sales (Walz, 2004). This economic injury, if it occurs at all, happens at very low frequency. Unless, of course, a compensation fund is available to reward its detection.

Looking further at the compensation issue, how would the benefits of biotech crops be factored in? A recent study found that with respect to the introduction of Bt maize in the Midwest (Illinois, Minnesota, Wisconsin, Iowa and Nebraska), growers that adopted those varieties over the past 14 years had gained $2.6 billion in economic returns, but in fact the non-adopters gained even more, $4.3 billion, from the reduced populations of corn borers that aided even (or particularly) organic growers at no additional cost (Hutchison et al., 2010). In some locations, such as for cotton in Arizona and papaya in Hawaii, organic production is only possible because of the pest and disease control effected by the conventional and biotech farmers who manage the vast majority of acres. How should that benefit be calculated into a fair compensation fund?

In addition to the organic industry, rejection of such crops or seeds can be a serious issue for those companies that export to countries that do not accept GE crops or where specific events are not yet approved. However, the seed industry deals with strict import standards all the time, particularly with respect to plant diseases and phytosanitary issues as well as genetic identity issues, and is able to work out procedures and organize production to meet the vast majority of those requirements. This is where co-existence is best represented and illustrated, and we should in fact be looking to the seed industry itself as a model for pragmatic mechanisms for co-existence.

Logical consequences

As a final exercise, I would like to point out some logical consequences of recent court cases and positions that have been taken with respect to gene flow and co-existence. I will start with principles that have been established or claimed by parties opposed to genetic engineering and follow them to their logical conclusion. For the sake of discussion, let's assume the following propositions and see where they can logically lead:

1. Farmers have a fundamental right to grow the seeds of their choice.
2. Organic farmers deserve protection in the form of large isolation zones and compensation funds to protect them and make them whole for any losses associated with GE presence.

3. Economic injury is a form of environmental impact, and is subject to National Environmental Protection Act (NEPA) jurisdiction. (The 9th Circuit Court rulings in requiring the USDA to do a full EIS for GT alfalfa and sugar beet appear to have established this principle.)
4. A given farm should be fully responsible and liable (based on strict liability) for anything that leaves that farm and causes economic injury to another farm.

If the Principle 1 is true, it should extend to growers of biotech crops also, and they should have the right to choose the seeds they want to plant. When available in a given crop, ~90% of them have chosen to grow biotech varieties because they offer greater economic value, whether in reduced inputs, higher yields, greater convenience, etc. They have made the choice and are receiving economic benefits from that choice. If a farmer in their vicinity decides to grow for the organic market, that farm must be certified by the NOP or its representative. Principle 2 states that as an organic grower, they would be entitled to a boundary zone, as we have seen enacted in the current permits for sugar beet seed production and were proposed as an alternative in the alfalfa ruling. Let's say that each organic farm is entitled to a 5-mile isolation zone free of GE crops. A circle of radius of 5 miles contains 78.5 square miles, or 50,265 acres (20,350 ha). Thus, one organic farm (of any size) would entail farmers in 50,000 surrounding acres being denied the opportunity to grow biotech varieties in order to enable the organic farm to meet its market goals. Those farmers who are now unable to grow the biotech crop of their choice will suffer economic injury equal to the enhanced value that they would have received from those crops relative to growing non-GE varieties. According to Principle 3, this is an economic injury and therefore an environmental impact by the organic farm on the surrounding area. As certification of organic farms and products is a government action by the NOP, it falls under NEPA. Thus, *a logical consequence of these three principles is that the NOP should be required to do an EIS before it certifies an organic farm in order to assess the potential impact of possible buffer areas or compensation funds on surrounding farms*. In keeping with the alfalfa and sugar beet court rulings, *the NOP should also refrain from any further certifications until the EIS is completed.*

Lest you think this is frivolous, the following quote is from the recent suit filed challenging the USDA's final ruling on GT alfalfa (Center for Food Safety v. Vilsack, 2011): "The effects that must be discussed in an EIS include, *inter alia,* the direct environmental impacts of the proposed action, the indirect effects of the proposed action, and the cumulative impacts of the proposed action. Direct effects are those "which are caused by the action and occur at the same time and place." 40 C.F.R. 1508.8(a). Indirect effects are those "which are caused by the action and are later in time or farther removed in distance, but are still reasonably foreseeable." 40 C.F.R. 1508.8(b). A cumulative impact constitutes the "impact on the environment which results from the incremental impact of the action when added to past, present, and reasonably foreseeable future actions regardless of what agency or person undertakes such other actions. Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time." 40 C.F.R. §1508.7." Certifying an individual organic farm might not be considered a major action, but certifying a number of them scattered over a given area could impact the entire region, as the example with the Willamette Valley showed. It seems inescapable that if organic farming becomes associated with mandatory buffer zones excluding neighboring farmers from choosing to grow biotech crops, then organic certification must require an EIS.

Similar logic holds for a compensation fund. Those in favor of a compensation fund propose that the developers of biotech crops should pay for the fund. Who would pay for a compensation fund for growers in the 50,000 acres surrounding an organic farm who are losing potential income due to their misfortune to be located within its mandated buffer zone? Given that economic benefits are being foregone to allow the organic farm to achieve its market goals, it seems logical that the organic farmer or the organic industry should be liable for creating such a fund and for compensating neighbors who are being disadvantaged by mandated buffers. Considering the specific case of the chard seed grower in Oregon, he wishes to be able to appeal to a compensation fund if his crop is cross-pollinated by GT sugar beets. However, chard pollen can blow as far as sugar beet pollen can. If Principle 4 above is established that farmers are strictly liable for anything that leaves their farm and causes economic injury to others, then it would seem equally fair that any sugar beet seed growers in the vicinity of the chard crop could claim injury if their sugar beet seeds exhibit evidence of out-crossing to chard. Such off-types can greatly reduce the value of hybrid seed, with proportionately large economic losses. Once legal precedents are established, and compensation funds are considered to be valid gene flow mitigation mechanisms, why should they not be applied in both directions?

Conclusions

Without trying to minimize the seriousness of the consequences of gene flow for specific growers and markets, I have tried to illustrate that we also have to examine the consequences of proposals and mechanisms for mitigating gene flow. Do I truly believe that the NOP should conduct an EIS for every organic farm or that the organic industry should establish a compensation fund for biotech growers? Not really, but neither do I believe that organic farmers should have the right to unilaterally establish buffer zones to protect themselves from gene flow or to seek compensation when the environment in which they choose to grow a specific crop prevents them from meeting certain self-imposed certification standards. The seed industry deals with these issues all the time without (in most cases) resorting to lawsuits. I think that society (and judges) should be cautious about extending environmental rights into the realm of economics and markets, as the effects of unintended consequences there have the potential to be much greater than have been demonstrated to date with biotech crops.

The solution to co-existence is really quite simple, and it comes down to pragmatic, achievable thresholds that can allow all segments of the industry to meet their market goals combined with a willingness to discuss and compromise. Zero tolerance standards and the ratcheting down of thresholds as testing methods improve is neither a credible nor reasonable path to co-existence. Zero tolerance thresholds are not a human or environmental safety issue, but solely a marketing issue, and as such, should not be a factor in regulatory decisions about the safety of biotech crops. Recent court cases that have extended greatly the right to be GE-free are establishing far-reaching precedents that could have major impacts on agriculture (Conko and Miller, 2010). Those who pursue such litigation should take a moment to ponder the potential consequences for their own supporters. It is a simple matter to turn those precedents around and employ them to the detriment of those who have established them. Surely co-existence and accommodation are better strategies than escalating legal warfare.

References

Brookes, G., and Barfoot, P. (2008). Global impact of biotech crops: socio-economic and environmental effects, 1996-2006. AgBioForum **11,** 21-38.

Brookes, G., and Barfoot, P. (2009). Global impact of biotech crops: income and production effects, 1996-2007. AgBioForum **12,** 184-208.

Center for Food Safety v. Vilsack. (2010). WL 3825699 (N.D. Cal., Sept. 28, 2010).

Center for Food Safety v. Vilsack. (2011). Complaint CV11-1310 (N.D. Cal. Mar. 18, 2011).

Conko, G., and Miller, H.I. (2010). The environmental impact subterfuge. Nat Biotech **28,** 1256-1258.

Economic Research Service. (2008). 2008 Organic Survey (http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Organics/).

Hutchison, W.D., Burkness, E.C., Mitchell, P.D., Moon, R.D., Leslie, T.W., Fleischer, S.J., Abrahamson, M., Hamilton, K.L., Steffey, K.L., Gray, M.E., Hellmich, R.L., Kaster, L.V., Hunt, T.E., Wright, R.J., Pecinovsky, K., Rabaey, T.L., Flood, B.R., and Raun, E.S. (2010). Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science **330,** 222-225.

McGinnis, E.E., Meyer, M.H., and Smith, A.G. (2010). Sweet and sour: a scientific and legal look at herbicide-tolerant sugar beet. The Plant Cell Online **22,** 1653-1657.

National Organic Coalition. (2011). Draft Proposal for USDA Monitoring, Mitigation, and Compensation Plan for GMO Contamination (http://www.nationalorganiccoalition.org/GEAlfalfa/ProposedCompensationPlan.pdf).

NOP Final Rule. (2000). 65 Fed. Reg. 80548, 80556 (Dec. 21, 2000).

Organic Seed Alliance. (2010). A Seed Saving Guide for Gardeners and Farmers (http://www.seedalliance.org/uploads/publications/Seed_Saving_Guide.pdf).

Stokstad, E. (2011). Can biotech and organic farmers get along? Science **332,** 166-169.

USDA-APHIS. (2010). Glyphosate-Tolerant Alfalfa Events J101 and J163: Request for Nonregulated Status Final Environmental Impact Statement—December 2010 (http://www.aphis.usda.gov/biotechnology/downloads/alfalfa/gt_alfalfa%20_feis.pdf).

Walz, E. (2004). Fourth National Organic Farmers' Survey: Sustaining Organic Farms in a Changing Organic Marketplace (Santa Cruz, CA: Organic Farming Research Foundation).

Gene flow from cultivated plants to wild/weedy/feral relatives

Allison A. Snow
Dept. of Evolution, Ecology, & Organismal Biology, Ohio State University, Columbus, 43210

Abstract

Concerns about gene flow from transgenic crops have prompted a great deal of research on the extent to which crop alleles can spread and persist in wild, weedy, and volunteer populations of free-living plants. In some cases, previous assumptions about the likelihood of gene flow to wild relatives have been revised. For example, pollen from creeping bentgrass seed nurseries can reach related plants that are >10 miles away, contrary to expectations (Watrud et al., 2004). Cultivars that are not highly domesticated (e.g., canola, clover, switchgrass) can give rise to naturalized, feral populations. Transgenic traits that enhance growth and reproduction have the potential to make free-living plants weedier and harder to manage. Therefore, a baseline understanding of the extent and consequences of gene flow from cultivated plants, whether transgenic or not, is essential for avoiding or mitigating unwanted consequences. To date, feral and volunteer canola populations that are resistant to commonly used herbicides are considered a nuisance, but few other problems related to transgene flow have been reported. When new yield-enhancing traits are introduced into a broader array of cultivated plants, additional research may be needed to avoid possible unwanted consequences of crop-to-wild gene flow.

General expectations about gene flow

In the US, crops such as corn, soybean, and cotton seldom occur near wild or weedy taxa that could hybridize with them, while other crops can easily interbreed with undomesticated relatives. In addition, many cultivated plants are capable of producing volunteer or naturalized populations when seeds or other propagules disperse to unmanaged habitats. Pollen-mediated gene flow from cultivated plants to their wild, weedy, or naturalized relatives has been demonstrated in species such as canola, rice, sorghum, wheat, oats, rye, sunflower, squash, radish, carrot, lettuce, raspberry, sugar beet, creeping bentgrass, and poplar (e.g., Ellstrand et al., 1999; Qwit et al., 2011). This is not surprising when wild relatives are primarily outcrossing, but even self-pollinating species such as weedy rice can hybridize with crops, albeit at a lower frequency (e.g., Rong et al,. 2006; Olguin et al., 2009). The distances over which pollen and seeds can travel vary greatly among species, and in many cases the full extent of rare, long-distance dispersal or extreme longevity are not known (e.g., Williams 2010).

Cultivars that can give rise to free-living volunteer populations include canola, carrot, rye, clover, and many grasses, shrubs, and trees (Gressel 2004; Ellstrand et al., 2010). For example, switchgrass (*Panicum virgatum*) is a relatively new crop that differs from wild populations by only a few generations of selection (Vogel and Burson, 2004). Callery pear (*Pyrus calleryana*) is an ornamental tree that recently became weedy after self-incompatible clones began cross-pollinating with each other (Culley and Hardiman, 2009). Many invasive species have become established after repeated planting for horticultural or landscaping purposes, with kudzu, cheatgrass, and water hyacinth representing well known examples.

Researchers have debated whether crop alleles are likely to survive in the wild, with some arguing that low hybrid fitness and/or linkage to domestication traits could severely hamper crop allele persistence (i.e., introgression). However, many crop-weed-wild gene pools are not discrete, but frequently mix and overlap as inter-breeding hybrid populations (e.g., Linder et al. 1998). Transgenes represent excellent markers for tracking the movement of genes from cultivars. Transgenes that confer resistance to two common herbicides, glyphosate and glufosinate, have been found in volunteer and feral populations of canola (*Brassica napus*), *via* both pollen- and seed-mediated gene flow (e.g., Hall et al. 2000; Kawata et al. 2009). Also, four years after transgenic *B. napus* hybridized with weedy *B. rapa* at two sites in Canada, transgenic resistance to glyphosate was detected in *B. rapa* (Warwick et al. 2008). In another well-studied example, the accidental escape of pollen and seeds from transgenic creeping bentgrass (*Agrostis stolonifera*) led to inadvertent gene flow to wild and naturalized populations in Oregon prior to regulatory approval (Watrud et al. 2004; Zapiola et al. 2008). These studies are consistent with the expectation that selectively neutral or beneficial transgenes may persist in crop volunteers and wild/weedy/naturalized populations.

Case study of weedy "wild radish"

My colleagues and I have been investigating questions related to gene flow in radish, sunflower, squash, rice, sorghum, and canola (http://www.biosci.ohio-state.edu/~asnowlab/home.html). In wild sunflower, for example, we discovered that a Bt transgene for insect-resistance could make crop-wild hybrids produce up to 50% more seeds per plant (Snow et al., 2003). In other studies, we used wild radish (*Raphanus raphanistrum*) as a tractable, model system to learn more about crop-to-wild gene flow and rapid evolution. Transgenes are expected to introgress into wild populations in the same manner as conventional crop alleles, under the same influences of the fitness effects of novel genes, linkage to other traits, and heterosis or outbreeding depression. Therefore, a great deal can be inferred about transgenes by studying "normal" crop-wild hybridization.

Initially, we found that F_1 crop-wild radish hybrids had greatly reduced fitness compared to wild plants, but their progeny multiplied quickly and soon evolved wild-type pollen fertility in experimental populations. Crop-specific alleles persisted in these populations for at least 15 years (Snow et al. 2010; ongoing research). In a related study, we allowed replicated wild *vs.* F_1 crop-wild hybrid radish populations to evolve for four generations and then compared their survival and fecundity in two common garden experiments - one in Michigan and one in California (Campbell et al., 2006). To our surprise, the relative fitness of crop-wild hybrids was strongly context-dependent. F_4 hybrids performed far better than wild plants in California, where weedy hybrid-derived populations have evolved spontaneously (Hegde et al., 2006), than in Michigan. These results are consistent with other evidence demonstrating that alleles from cultivated radish can persist in hybrid populations and may have contributed to the success of a common agricultural and roadside weed in California.

Implications for risk assessment

Many agricultural weeds have large populations, long-lived seed banks, and extensive pollen- and seed-mediated gene flow, all of which can facilitate the spread and persistence of crop alleles. Therefore, if the crop and its wild relative co-occur, share flowering periods, and produce fertile F_1 hybrids, it seems likely that neutral or beneficial crop alleles could persist in free-living populations. Transgenic traits such as insect resistance due to Bt genes have been shown to enhance fitness in wild sunflower and weedy rice in pre-commercial, closely-monitored experiments (Snow et al., 2003; Yang et al., 2011). Likewise, transgenic virus resistance greatly increases seed production in wild/weedy squash (Laughlin et al. 2009; Sasu et al., 2009).

Fitness comparisons between transgenic *vs*. non-transgenic hybrid progeny from the same genetic background provide a useful starting point for evaluating potential problems that could result from gene flow. These experiments should be carried out in a range of environments and locations to test for genotype-by-environment interactions (Campbell et al., 2006). Genetically engineered hybrids that are more competitive, survive better, or produce more seeds than their nontransgenic counterparts merit further study to determine what limits the abundance and distribution of hybridizing populations. In other words, could release from biotic or abiotic stresses due to the acquisition of transgenes allow rapid population growth and/or invasion of new habitats? In the future, we expect that research on the extent and consequences of gene flow will continue to be in demand, especially when new, yield-enhancing transgenic traits and new, semi-domesticated bioenergy crops are proposed for commercialization.

References

Campbell L.G., A.A. Snow, and C.E. Ridley. 2006. Weed evolution after crop gene introgression: greater survival and fecundity of hybrids in a new environment. Ecol. Letters 11:1198–1209.

Culley, T.M., and N.A. Hardiman. 2009. The role of intraspecific hybridization in the evolution of invasiveness: A case study of the ornamental pear tree *Pyrus calleryana*. Biological Invasions 5: 1107-1119.

Ellstrand, N.C., H.C. Prentice, and J.F. Hancock. 1999. Gene flow and introgression from domesticated plants into their wild relatives. Ann. Rev. Ecol. Syst. 30:539-563.

Ellstrand, N.C., Heredia, S.M., Leak-Garcia, J.A., Heraty, J.M., Burger, J.C., et al. 2010. Crops gone wild: evolution of weeds and invasives from domesticated ancestors. Evol. Applications 3:494-504.

Gressel, J. 2005. Crop ferality and volunteerism. CRC Press, Taylor & Francis Group, LLC, Boca Raton, FL. ISBN 0-8493-2895-0.

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

Hegde S.G., J. D. Nason, J.M. Clegg, and N.C. Ellstrand. 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. Evol. 60, 1187–1197.

Kawata, M., K. Murakami, and T. Ishikawa. 2009. Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. Env. Science and Pollution Research 16:120-126.

Laughlin, K., A.G. Power, A. A. Snow, and L. J. Spencer. 2009. Environmental risk assessment of genetically engineered crops: potential fitness-related effects of virus-resistance transgenes in wild squash populations (*Cucurbita pepo*). Ecological Applications 19:1091-1101.

Linder, C.R., I. Taha, G.J. Seiler, A.A. Snow, and L.H. Rieseberg. 1998. Long-term introgression of crop genes into wild sunflower populations. Theoretical and Applied Genetics 96:339-347.

Olguin, E.R.S., G. Arrieta-Espinoza, J.A. Lobo, and A.M. Espinoza-Esquivel. 2009. Assessment of gene flow from a herbicide-resistant indica rice (*Oryza sativa* L.) to the Costa Rican weedy rice (*Oryza sativa*) in tropical America: factors affecting hybridization rates and characterization of F(1) hybrids. Transgenic Research 18:633-647.

Rong, J., B.-R. Lu, Z. Song, J. Su, A.A. Snow, et al. 2006. Dramatic reduction of crop-to-crop gene flow within a short distance from transgenic rice fields. New Phytologist 173: 346-353.

Sasu, M.A., M.J. Ferrari, D.L. Du, J.A. Winsor, and A.G. Stephenson. 2009. Indirect costs of a nontarget pathogen mitigate the direct benefits of a virus-resistant transgene in wild *Cucurbita*. Proc. Natl. Acad. Sci. 106:19067-19071.

Snow A.A., D. Pilson, L.H. Rieseberg, M.J. Paulsen, N. Pleskac, M.R. Reagon, D.E. Wolf, and S.M. Selbo. 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecol. Appl. 13, 279–286.

Snow, A. A., T.M. Culley, L.G. Campbell, P. M. Sweeney, S. G. Hegde, and N.C. Ellstrand. 2010. Long-term persistence of crop alleles in weedy populations of wild radish **(***Raphanus raphanistrum*). New Phytologist 186:537-548.

Kwit, C., H.S. Moon, S.I. Warwick, and C.N. Stewart. 2011. Transgene introgression in crop relatives: molecular evidence and mitigation strategies. Trends in Biotechnology 29:284-293.

Vogel, K.P., and B. Burson. 2004. Breeding and Genetics. p. 51-96. In L.E. Moser, L. Sollenberger, and B. Burson (ed.). Warm-season (C_4) grasses. ASA-CSSA-SSSA Monograph. Madison, WI.

Warwick,S.I., A. Légère, M.J. Simard, and T. James. 2008. Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. Mol. Ecol. 17, 1387–1395.

Watrud, L.S., E.H. Lee, A. Fairbrother, C. Burdick, J.R. Reichman, M. Bollman, M. Storm, G. King, and P.K. Van de Water. 2004. Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. Proceedings of the National Academy of Sciences 101:14533-14538.

Williams, C.G. 2010. Long distance pine pollen still germinates after meso-scale dispersal. Am. J. Bot. 97:846-855.

Yang, X., X. Hiu, F. Wang, J. Su, A.A. Snow, and B.-R. Lu. 2011. Transgenes for insect resistance reduce herbivory and enhance fecundity in advanced generations of crop-weed hybrids of rice. Evolutionary Applications, doi:10.1111/j.1752-4571.2011.00190.x

Zapiola M.L., Campbell C.K., Butler M.D., Mallory-Smith C.A. 2008. Escape and establishment of transgenic glyphosate-resistant creeping bentgrass *Agrostis stolonifera* in Oregon, USA: a 4-year study. J. Appl. Ecol. 45, 486–494.

Potential for Persistence of Genes in the Environment

Authors: Joseph M. DiTomaso[1]*, Jacob N. Barney[2], J. Jeremiah Mann[1] and Guy B. Kyser[1]
[1]University of California, Davis, 1 Shields Ave, Davis, 95616; [2]Virginia Tech, Blacksburg, VA 24061

Abstract

The global energy portfolio is projected to become increasingly reliant on bio-based products. Biofuel crops will likely comprise many species globally, and will be selected to require minimal inputs, tolerate marginal growing conditions, and exhibit rapid growth rates. These desirable agronomic traits also characterize many of our worst invasive species. Many of the candidate biofuel crops are already known invasive or noxious species in portions of their non-native range. Others demonstrate a high likelihood of becoming invasive in the target cropping region. Thus, it is critical to understand the characteristics that lead to escape and invasiveness of proposed biofuel species, and to develop and implement mitigation protocols prior to large scale adoption. Such protocols will contribute to sustainable production systems, as well as minimizing the risk of future invasion that may offset the benefits of bio-based energy crops.

Biology of Invasive Plants

Executive Order (EO 13112) established in 1999 defines an invasive species as an "alien (non-native) species whose introduction does or is likely to cause economic or environmental harm or harm to human health." Harmful invasive species cost the global economy trillions in lost revenue and management costs annually (Pimentel et al. 2000), and are cited as the second greatest threat to biodiversity (Mack et al. 2000). Invasive plants are well recognized to reduce native species biodiversity, alter biogeochemical cycles, modify food webs, transform natural disturbance regimes, increase flooding and fire frequency and intensity, and reduce deep soil water storage.

While a small fraction of the total number of introduced plants into the United States have caused significant harm, many have neutral consequences while many others provide tremendous benefits to society, including food, fiber, construction materials and ornamental species. Unfortunately, some introduced plants were established with the intention of providing great economic benefits, only to escape and cause significant economic or ecological damage. Examples of such species include kudzu (*Pueraria montana* var. *lobata*), johnsongrass (*Sorghum halepense*), saltcedar (*Tamarix ramosissima* and hybrids), giant reed (*Arundo donax*), and water hyacinth (*Eichhornia crassipes*). Kudzu alone now infests an estimated 40 million ha in the southeastern U.S. where it smothers native ecosystems, crowds out desirable species, and reduces land value. The characteristics that lead to the escape and invasion of introduced plants have been widely studied among ecologists. In addition, while species characteristics can certainly lead to increased probability of invasion, habitats vary in their susceptibility to invasion, which is often a function of colonization pressure (i.e., the number of species introduced to a location) and propagule pressure (i.e., the number of individuals and events introduced to a single location).

It is important to recognize that many of the traits considered desirable for proposed biofuel species are also those that can be found in some of the most invasive species in the country.

Traits such as a perennial growth form; rapid and high aboveground biomass production; tolerance to drought, low fertility, or high salinity soils; competitiveness with other vegetation; and a lack of resident pathogen or insect pests that keep populations in check contribute to both a species' ability to yield biofuel and become invasive (DiTomaso et al. 2010). Considering the potential scale of biofuel cultivation, which is estimated at 1.5 billion ha by 2050 globally (Field et al. 2008), there will be ample opportunity for biofuel crops to be introduced into environments in which they could persist and adversely interact with natural or managed ecosystems.

One aspect of integrating biomass-based crops into our energy portfolio sustainably will be to utilize crops that present an acceptably low risk of becoming invasive. Currently, there is no prescription for identifying which plants will become invasive, however all introduced species present some level of invasion risk, so it is not only important to identify that risk, but also to mitigate against the known hazards.

Evaluating the Invasive Risk of Biofuel Crops

Several types of evaluations are available to determine the potential risk and performance of biofuels crops in various landscapes or conditions (Barney et al. 2011). Among these qualitative and quantitative studies include:

1. Perform risk assessment using a science-based protocol to determine invasion potential
2. Determine the potentially invasible range using climate-matching analyses under various assumptions (e.g., drought tolerance) and scenarios (e.g., irrigation, climate change)
3. Evaluate environmental tolerance (e.g., soil moisture stress) of target biofuel crops
4. Quantify invasibility of susceptible habitats (e.g., riparian areas, rangeland)
5. Perform propagule biology studies – seeds, stem and rhizome fragments
6. Assess hybridization potential with related native and non-native taxa
7. Evaluate competitive interactions with desirable species

We performed these evaluations for two leading perennial grass biofuel candidates, switchgrass (*Panicum virgatum*) and giant miscanthus (*Miscanthus* x *giganteus*). Initially, we screened both species through the most widely adopted weed risk assessment (WRA) protocol designed for Australia and New Zealand (Pheloung et al. 1999). The WRA results for miscanthus in the United States yielded an "accept" rating. The relatively minor risk of invasion from miscanthus is attributed primarily to the lack of seed production (Barney and DiTomaso 2008). In contrast, switchgrass was found to have a risk of potential invasive in California, unless sterility is introduced, and then it was considered acceptable with low potential for invasiveness.

In another study, we determined the potential range of both switchgrass and miscanthus using CLIMEX software. This climate matching model estimates the potential range for each species utilizing the distribution and abundance of known populations. The evaluation of both switchgrass and miscanthus were conducted at a global level (Barney and DiTomaso 2011) and switchgrass was also evaluated at a national scale (U.S.), as well as at the regional level (western U.S.) (Barney et al. 2010a). Such analyzes can predict where the two species are likely to survive with and without irrigation. Areas requiring irrigation to survive and persist have the potential to become invasive only in riparian or perennially wet areas.

To evaluate the environmental tolerance of the two biofuel species, we assessed the germination or vegetation propagule growth, establishment, performance, and reproductive potential of miscanthus and four common switchgrass accessions at various levels of soil moisture availability (moisture deficit to flooded) in the greenhouse. Our results indicated that while switchgrass is not well adapted to prolonged drought (Barney et al. 2009), it was far more tolerant to low soil moisture compared to miscanthus (J. Mann, unpublished data). Furthermore, the results support the findings from the climate matching model indicating that both species were well adapted to saturated moisture conditions. The results of these studies led to a field study evaluating the survival, establishment, and naturalization potential of switchgrass and miscanthus in non-native riparian and dryland habitats under varying levels of resource availability and competition over two years. The results again supported the greenhouse findings, indicating that neither species, particularly miscanthus, was capable of surviving in Mediterranean climates without summer rainfall, but both species performed well with seasonal moisture along the stream bank. Furthermore, the high resource environment without competition was more invasible (capable of supporting an introduced propagule) than a relatively lower resource environment with an intact riparian community (Barney et al., unpubl. data).

From additional greenhouse experiments, we showed that in the case of switchgrass, the primary dispersal propagule was seeds, with little regeneration from shoot fragments. In contrast, giant reed , an invasive plant also being considered for biomass production, readily produces new shoots from almost every stem node. Similarly, miscanthus neither produces viable seeds nor new shoots from stem nodes later in the year (Mann, unpubl. data). Both species produce rhizomes which can also serve as a source of propagules. However, this is only possible in a riparian system where flooding can lead to soil erosion and rhizome dispersal downstream. Such an event is very unlikely to occur under agronomic cultivation. Thus, the threat of propagule escapes from sterile forms of miscanthus and, yet to be developed, sterile switchgrass is extremely low. In contrast, giant reed also does not produce viable seed, but the dispersal of stem fragments has led to its widespread invasion in California and Texas.

As a final consideration, miscanthus is sterile and even if it were fertile, there is no potential for hybridization with native species, as the genus *Miscanthus* is not represented in the native flora of the U.S. It is possible, though improbably, that a fertile form of giant miscanthus could hybridize with other escaped populations of non-native *M. sinensis* or *M. sacchariflorus*. In contrast, there are several native species of *Panicum*, of which switchgrass is one of them. However, there is no record of *Panicum virgatum* hybridizing with any other species of *Panicum*, either native or non-native.

Mitigating the Risk Potential of Biofuel Crops

While riparian areas in the western U.S. are capable of supporting both switchgrass and miscanthus, the conditions by which they are likely to become invasive are quite narrow. Thus, we believe that with appropriate screening and cogent application of mitigation strategies at various points along the biofuel supply chain, expedient biofuel crop implementation can be efficient and environmentally safe.

Our recommendations (Barney and DiTomaso 2010b, Barney et al. 2011) include that biofuel crop developers make every effort not to utilize known invasive species. In addition, propagule dissemination and movement prior to cultivation and in the stages of harvest, transport and storage should be minimized, particularly in the vicinity of susceptible agricultural or environmental ecosystems. Thus, growers, transporters, and processors should follow specific guidelines that minimize or eliminate unintentional dispersal of biofuel crop propagules, and maintain biofuel crops within production and processing boundaries. Finally, prior to commercialization and wide-scale cultivation of biofuel crops, eradication protocols need to be developed to rapidly respond to escaped plants or populations, reclaim abandoned production fields, or facilitate rotation to other crops.

The risk of invasiveness with biofuel crops can be quantified through a series of studies combining risk assessment, biological and ecological studies, niche modeling, and crop management in each target cropping region. Such studies conducted in parallel with agronomic trials should not be overly burdensome to developers, growers, refiners, or regulators, but will require their collaboration to ensure that biofuel crops are produced sustainably with an acceptably low risk of invasiveness.

References

Barney, J.N. and J.M. DiTomaso. 2008. Non-native species and bioenergy: are we cultivating the next invader? BioScience 58:64-70.

Barney, J.N. and J.M. DiTomaso. 2010a. Bioclimatic predictions of habitat suitability for the biofuel switchgrass in North America under current and future climate scenarios. Biomass and Bioenergy 34:124-133.

Barney, J.N. and J.M. DiTomaso. 2010b. Invasive Species Biology, Ecology, Management and Risk Assessment: Evaluating and Mitigating the Invasion Risk of Biofuel Crops, Pages 263-284, in Biotechnology in Agriculture and Forestry, Andrea Schlitzberger, ed. Springer Publ. Co., New York.

Barney, J.N. and J.M. DiTomaso. 2011. Global climate niche estimates for bioenergy crops and invasive species of agronomic origin: Potential problems and opportunities. PLoS ONE 6(3): e17222. doi:10.1371/journal.pone.0017222

Barney, J.N., J.J. Mann, G.B. Kyser, E. Blumwald, and A. Van Deynze, and J. M. DiTomaso. 2009. Tolerance of switchgrass to extreme soil moisture stress: ecological implications. Plant Science 177:724-732.

Barney, J.N., J. J. Mann, G. B. Kyser, J. M. DiTomaso. 2011. Mitigating the invasive risk potential of biofuel crops, Topics in Canadian Weed Science 7: (In Press)

DiTomaso, J.M., J.K. Reaser, C.P. Dionigi, O.C. Doering, E. Chilton, J.D. Schardt, and J. N. Barney. 2010. Biofuel vs. Bioinvasion: Seeding Policy Priorities. Environmental Science and Technology 44:6906-6910.

Field, C. B., J.E. Campbell and D.B. Lobell. 2008. Biomass energy: the scale of the potential resource. Trends Ecol. Evol. 23 (2):65–72.

Mack, R.N. 2000. Cultivation fosters plant naturalization by reducing environmental stochasticity. Biol Invasions 2:111–122.

Pheloung, P.C., P.A. Williams and S.R. Halloy. 1999. A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. Journal of Environmental Management 57: 239-251.

Pimentel, D., L. Lach, R. Zuniga and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience 50:53–65.

Gene Movement from Grasses

Carol Mallory-Smith
Oregon State University, 3050 SW Campus Way, Corvallis 97331

Abstract

Grasses pose one of greatest challenges for managing gene containment. There are an estimated 10,000 grass species many of which are economically and environmentally important. These species include food and feed cereal grain crops and turf and forage grasses. In addition, some grasses are already being used and others are being considered for biofuel production. The diversity of grasses and their biology make it impossible to make simple or general predictions about how and when gene movement will occur. Gene movement for the same species may be low under some conditions and high under other conditions. One component of the gene movement discussion that is not well understood by regulators or other non-agriculturalists is how crop production practices influence gene movement. The one certainty based on past experience is that there will be gene movement between species via pollen, seed, or vegetative propagules.

Introduction

Gene movement in grass species is complicated and variable. Cereal grain crops have a long history of breeding. Resources from both public and private sectors have been committed to understanding their genetics and reproduction in order to increase yield and quality. Therefore, gene movement from these crops is much more predictable than from turf and forage grasses where resource commitment has been much less. In addition, the biology, genetics and breeding systems of the turf and forage grasses are generally much more complicated than the cereal grains. The cereal grain crops have fewer compatible relatives, simpler reproduction systems, and are annuals.

Cereal Grain Crops. The major USA cereal grain crops, in order of acres grown, are corn (*Zea mays*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor ssp. bicolor*), and rice (*Orzya sativa*). These species vary in their pollination biology and the number of compatible species that occur in the production systems (see reviews by Mallory-Smith and Zapiola 2008; Mallory-Smith and Sanchez 2011). Corn is a cross pollinated species, but has no compatible or wild or weedy relatives in the USA, so gene movement will only occur with other cultivars of corn or through seed mixing in the supply distribution chain. Wheat, sorghum, and rice are predominately self-pollinating but do outcross. Gene movement occurs between wheat cultivars and with the compatible species, jointed goatgrass (*Aegilops cylindrica*). The hybrids are male sterile with low female fertility. Seed admixture occurs in the wheat supply chain. Sorghum is compatible with several other crops species including forage sudangrass (*S. biocolor* ssp. drummondii) as well as the weed species johnsongrass (*S. halpense*) and shattercane (*S. bicolor* spp. *arundinaceum*). Gene movement within the sorghum complex will occur but is difficult to predict. Nontransgenic herbicide resistant sorghum is under development and the movement of the resistance trait to compatible species should be expected to occur quickly following its commercialization. Rice will hybridize with red rice, a conspecific weed species. Movement of the gene that confers resistance to the imidazolinone herbicides has been documented from wheat to jointed goatgrass and from rice to red rice. Regulated genes have been found in USA corn and rice supplies and will continue to be found in cereal grains as more are commercialized.

Turf and Forage Grasses. Turf and forage grasses are not highly domesticated although there is a more concerted effort to breed turf type grasses. A 2004 report listed 78 perennial grass species for turf or forage use (Cast 2004). In addition to these species, there are annual grass species used for turf and forage. Grasses vary in adaptation to environmental conditions with a major distinction between cool and warm season grasses with graduation between the two environments. Common cool season grasses include tall fescue (*Festuca arundinacea*), perennial ryegrass (*Lolium perenne*), and Kentucky bluegrass (*Poa pratensis*); common warm season grasses include Bermudagrass (*Cynodon dactylon*), zoysiagrass (*Zoysia japonica*) and seashore paspalum (*Paspalum vaginatum*). There is also variation in response to of these grasses abiotic (e.g. drought or salinity) and biotic (disease or insect) stresses.

Reproduction systems vary among these grass species but most reproduce sexually and are self-incompatible with very low levels of self-pollination. Pollen movement is usually via wind. The requirement for cross pollination to produce seed obviously increases gene movement in the environment. Outcrossing guarantees that the populations maintain genetic variability. Asexual reproduction can occur through apomixes (nonsexual production of seed) or through vegetative propagules. Even though apomixes is considered asexual reproduction, there are different outcomes of apomixes which result in progeny that are not clones of the parent and which have chromosome variation. Apomixes increases the potential for a species to survive during unfavorable conditions. Generally any species that reproduces via apomixes also will reproduce sexually. Ploidy levels and chromosome numbers vary within some species. Interspecific and intergeneric hybrids occur with many of the grass species. For example, perennial ryegrass will cross with the two annual species *L. multifloum* and *L. rigidum*. The *Lolium* species also will cross with tall fescue and meadow fescue (*Festuca pratensis*). The resulting hybrids vary in fertility depending on the cross.

Two examples of grass species that are currently of major concern for gene movement are transgenic glyphosate resistant creeping bentgrass (*Agrostis stolonifera*) and Kentucky bluegrass. The difference in the transformation methods used for these two species resulted in APHIS regulation of creeping bentgrass but not Kentucky bluegrass.

Creeping bentgrass is a cool season grass widely used on golf courses because it can withstand low mowing. Creeping bentgrass is an obligate outcrossing species which produces both intrageneric and intergeneric hybrids (Warnke 2003). It also reproduces vegetatively via stolons. In Oregon, the movement of the glyphosate resistance transgene from creeping bentgrass to compatible species while under regulated status has been well documented (Watrud et al. 2004; Reichman et al. 2006; Zapiola et al. 2008). More than 8 years after the seed production fields near Madras, OR, were removed there are transgenic plants still present and reproducing (personal observation). In 2010, transgenic creeping bentgrass was identified in Eastern Oregon where no permit was issued for production. It is believed that the infestation is the result of seed movement from seed production fields located across the Snake River in Idaho. Those seed production fields were removed at least 5 years ago. This infestation is large, covering many miles of irrigation canals and ditches (personal observation). Glyphosate-resistant creeping bentgrass still has not been deregulated by APHIS.

Kentucky bluegrass (*Poa pratensis*) is one of the most economically important and widely planted forage and turf grass species. It is also found in many different habitats such as roadsides, rangelands, meadows, and mountains. According the Funk (2000), 90% of the Kentucky bluegrass that is found in pastures, highways, and on rangelands was not intentionally sown, which provides a source of genetic variability and also is an indication of its ability to survive outside of cultivation. Kentucky bluegrass is believed to have been introduced to the USA from Europe but distributed circumpolar. The announcement that a nonregulated transgenic glyphosate-resistant Kentucky bluegrass will be commercialized raised concerns within the seed production industry and ecologists about movement of the trait because Kentucky bluegrass readily crosses with several species. Kentucky bluegrass reproduces by cross pollination, by apomixes, which can produce four different aberrant progeny types, and by rhizomes. It also has variable ploidy levels and chromosome numbers (x=7; 2n =28 to 154) (Funk 2000). The species can absorb entire genomes or other *Poa* species. Hybridization with other *Poa* species makes identification and classification nearly impossible (Huff 2003)

Summary

Grasses will continue to pose gene movement challenges. The complicated reproduction and widespread use of turf and forage grasses increases the management issues for gene containment. The perennial nature of most turf and forage grasses complicates monitoring of the gene once it is released. The unpredictability of hybridization of these species also warrants extra caution before the release of plants with traits that increase fitness or ecological amplitude. The cereal grains, although better understood genetically, still pose problems of gene movement to compatible species either crop or weed. There needs to be a stronger emphasis on understanding not only the biology of grass species but also of their production systems which vary, and therefore, influence gene movement.

References

CAST (Council for Agricultural Science and Technology). 2004. Biotechnology-derived perennial turf and forage grasses: criteria for evaluation. eds. M. Kenna and W.K. Hallman. Special Publication 25.

Funk, C.R. 2000. Long live Kentucky bluegrass, the king of grasses! Diversity 16:26-28.

Huff, D. 2003. Kentucky bluegrass. *In* Turfgrass Biology, Genetics, and Breeding. John Wiley & Sons. Hoboken, NJ pg. 27-38.

Mallory-Smith, C. and E. Sanchez Olguin. 2011. Gene flow from herbicide-resistant crops: it’s not just for transgenes. J. of Ag and Food Chem. 59:5813-5818.

Mallory-Smith, C.A., and M.L. Zapiola. 2008. Gene flow from glyphosate-resistant crops. Pest Management Sci. 64:428-440.

Reichman, J.R., L.S. Watrud, E.H. Lee, C.A. Burdick, M.A. Bollman, M.J. Storm, G.A. King, and C.A. Mallory-Smith. 2006. Establishment of transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) in nonagronomic habitats. Molecular Ecol. 15:4243-4255.

Warnke, S.E. 2003. Creeping Bentgrass. *In* Turfgrass Biology, Genetics, and Breeding. John Wiley &Sons. Hoboken, NJ pg. 175-186.

Watrud, L.S., E.H. Lee, A. Fairbrother, C. Burdick, J.R. Reichman, M. Bollman, M. Storm, G. King, and P.K. Van de Water. 2004. Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. PNAS 101:14533-14538.

Zapiola, M.L., C. Campbell, M. Butler, and C.A. Mallory-Smith. 2008. Escape and establishment of glyphosate-resistant creeping bentgrass (*Agrostis stolonifera*) in Oregon, USA: a 4-year study. J. of Applied Ecology 45:486-494.

Quantifying the Area-wide Dispersal Patterns of Honeybees in Commercial Alfalfa Fields

James R. Hagler[1*], Shannon Mueller[2], Larry R. Teuber[3], and Allen Van Deynze[3]

[1]Arid Land Agricultural Research Center, USDA-ARS, 21881 North Cardon Lane, Maricopa, AZ, USA 85138

[2]University of California Cooperative Extension, 1720 South Maple Avenue, Fresno, CA, USA 93702

[3]University of California, Department of Plant Sciences, Mail Stop 1, One Shields Avenue, Davis, CA, USA 95616

Abstract

A study was conducted to examine the foraging range of honey bees in an agroecosystem dominated by alfalfa seed production fields. Honey bee self-marking devices were attached to hives located within nine different apiaries. The foraging bees exiting each apiary location were uniquely tagged so that the apiary of origin and the distance traveled by field-collected bees could be measured. Honey bee self-marking devices were installed on approximately 13% of the total hives located within the research area. The frequency of field-collected bees possessing a distinct mark was similar, averaging about 14%. Over 12,000 bees were collected from the various alfalfa fields on seven sampling dates over the course of the two year study. The distances traveled by marked bees ranged from a minimum of 148 feet to a maximum of 3.7 miles. On average, marked bees dispersed a half mile from their apiary of origin and the recovery rate of marked bees decreased exponentially as the distance from the apiary increased. Ultimately these data were used to identify the extent of pollen-mediated gene flow from Roundup Ready® (RR) to conventional alfalfa.

Introduction

Alfalfa seed producers often depend on honey bees or leafcutter bees for pollination services. Specifically, alfalfa is a cross-pollinated, perennial crop that requires bees to “trip” the flowers in order to release pollen for seed production. Hence, knowledge of pollinator foraging behavior and the extent of pollen-mediated gene flow between commercial alfalfa seed production fields is vital to establishment of stewardship guidelines. The influence of pollinator species and environment will vary with location. In California, alfalfa is grown for both forage and seed over a relatively long production season. Therefore, these growers often use honey bees to pollinate their crops. In the Pacific Northwest, growers rely primarily on the alfalfa leafcutting bee to pollinate their seed crops. Each of these pollinating insects has foraging characteristics that influence gene flow. Honey bees can forage many miles from their hive and will visit a wide variety of plant species in search of floral rewards. Nectar-collectors far outnumber pollen-collectors in the honey bee population and they can gather nectar without tripping the flower and releasing pollen. The leafcutting bee has a shorter flight range than the honey bee and all of the females in the population (≈ 30%) gather both pollen and nectar to provision their nests. They have a much narrower range of plant types from which they collect nectar and pollen when compared to honey bees. The differences in foraging behavior between these pollinating insects result in differences in potential gene flow from fields where they are present.

Our study aimed to quantify the area-wide honey bee dispersal patterns throughout a 5.9 square mile agroecosystem that was dominated by seed production fields containing Roundup Ready (RR) and conventional alfalfa. To accomplish this goal, we used a novel honey bee self-marking system. Honey bee self-marking devices loaded with various distinct powdered markers were placed at the entrances of hives located in nine apiaries throughout the study area (Hagler et al. 2011a). The bees exiting each apiary were uniquely marked so that the apiary of origin and the distance traveled by the marked (field-collected) bees could be pinpointed. Ultimately these data were correlated with seed harvest data to identify the extent of pollen-mediated gene flow from RR to conventional alfalfa (see Teuber et al., herein).

Methods

The two year study was conducted in an alfalfa seed production area located in Fresno County, CA during alfalfa bloom. The area contained seven alfalfa fields from which foraging honey bees were collected. The seven fields included one 318 acre RR transgenic herbicide-tolerant seed production field, four small 1.8 acre conventional alfalfa seed production fields, one 240 acre conventional alfalfa seed production field, and one 55 acre conventional alfalfa seed production field (see Hagler et al., 2011a for details). The four small conventional fields were strategically planted in an equidistant linear fashion between the RR field and the 240 acre conventional field to serve as a "bridge" between the two types of commercial alfalfa seed fields. At the onset of alfalfa bloom, hundreds of commercial honey bee colonies were placed by beekeepers at strategic locations in the vicinity to provide pollination services for the alfalfa growers. A honey bee marking device was installed at the entrance of 112 colonies (≈ 13% of the colonies in the vicinity). Each alfalfa field, depending on its size, contained one to six 1.8 acre honey bee collection sites. Honey bees were collected from these sites using a standard sweepnet and taken back to the lab and analyzed for the presence of the various marks. Detailed descriptions of the honey bee marking device, marking scheme, sampling plan, mark detection protocols, and data analysis are given in Hagler et al. (2011a and 2011b).

Results and Discussion

There were seven sampling dates over two seasons with a total of 12,266 bees collected. Using the information recorded for each positively marked bee, the apiary of origin and flight distance to point of capture could be identified. The distance bees traveled ranged from 148 feet to 3.7 miles. The vast majority of marked bees were collected from the apiaries closest to the sampling site within that field. Of most interest in this study was the foraging distance of honey bees from apiaries adjacent to the RR field. One of the objectives of this study was to correlate the honey bee foraging distance with the percent gene flow in the study area. Similar to data presented on gene flow (see Teuber et al., herein), the recovery rate of marked bees decreased exponentially as the distance from the apiary of origin increased.

Although honey bees are known to fly great distances, the abundance of blooming alfalfa and lack of highly attractive alternative crops in the area limited the foraging distances observed during this study. Even when honey bees are found to forage at great distances, gene flow is not guaranteed. The bee needs to be carrying the pollen, successfully transfer it to a tripped flower where it then fertilizes the ovule and develops into a mature, viable seed.

In summary, understanding gene flow mediated by honey bee (and other pollinators) pollen dispersal is crucial for developing strategies to minimize adventitious presence of genetic traits and to maximize seed purity. The data described here on the foraging range of honey bees, coupled with analysis of the seed harvested from the study site (see Teuber et al., herein), will help establish isolation requirements to ensure genetic purity of alfalfa seed. The novel honey bee marking methodology can be used for future studies on area-wide dispersal of honey bees and leafcutter bees.

References

Hagler, J.R., S. Mueller, L.R. Teuber, S.A. Machtley, and A. Van Deynze. 2011a. Foraging range of honey bees, *Apis mellifera*, in alfalfa seed production fields. Journal of Insect Science, *In Press.*

Hagler, J.R., S. Mueller, L.R Teuber, A. Van Deynze, and J. Martin. 2011b. A method for synchronous and distinct marking of honey bees, *Apis mellifera* originating from multiple apiary locations. Journal of Insect Science, *In Press.*

Gene Flow in Commercial Alfalfa Fields and Implications to Isolation and Seed Certification

Larry R. Teuber[1*], Shannon Mueller[2], James R. Hagler[3], and Allen Van Deynze[1]

[1]University of California, Department of Plant Sciences, Mail Stop 1, One Shields Avenue, Davis, CA, USA 95616

[2]University of California Cooperative Extension, 1720 South Maple Avenue, Fresno, CA USA 93702[3]

[3]Arid Land Agricultural Research Center, USDA-ARS, 21881 North Cardon Lane, Maricopa, AZ, USA 85138

Abstract

Alfalfa (*Medicago sativa* L.) is a perennial species with an out-crossing rate of between 67 and 77 percent. Pollen is primarily vectored by bees. An accurate understanding of the frequency with which foreign pollen results in viable seed set is critical to establishing appropriate mechanisms for isolation and maintenance of genetic purity. Studies were conducted to determine the frequency of pollen mediated gene flow under commercial seed production conditions with either honey bees (*Apis mellifera*) or a combination of honey bees and alfalfa leaf cutter bees (*Megachile rotundata)* as pollinators. A 318 acre commercial seed production field with the Roundup Ready (RR) trait conferring Roundup® herbicide tolerance was used as a pollen-marking tool. Study site/trap plots (1.8 acres) were established between 165 ft and 5 miles. Approximately 1.2 million seedlings were evaluated for the presence of the RR trait from these study sites during each of two production years. There was a logarithmic decrease in gene flow between 165 ft and 3 miles from the RR source field. Gene flow averaged 1.67% at 165 ft, 0.22 percent at 1 mile, and was not detectable at 5 miles. These results are being utilized by seed certification agencies to develop isolation distances, and mapping and stewardship programs to be employed during the production of seed for markets that are sensitive to the presence of transgenic traits.

Introduction

Alfalfa is an outcrossing species with an out crossing rate of between 67 and 77 percent (Brown and Bingham, 1991; Knapp et al., 1993). Outcrossing rate, gene flow, pollinator behavior, and cultural conditions are all very important components of proper management during commercial alfalfa (*Medicago sativa* L.) seed production. There have been many studies conducted over the past 50 years attempting to understand these factors and to apply the results to commercial seed production practices and regulations. Unfortunately studying these factors has been particularly difficult because of the diversity that exists in production environments, cultivar characteristics, differences in pollinators, and lack of simply inherited and easily evaluated genetic markers.

Recent advances in genetic technology have both 1) highlighted the need to understand the dynamics of gene flow in order to maintain the varietal purity of both conventional and genetically enhanced cultivars and; 2) provided traits that are stable and can be easily assessed. Such traits can be used as genetic markers in conducting gene flow studies. Recent studies by Forage Genetics (2001) and St Amand et al. (2000) have used these techniques to study gene flow during seed production in Idaho with alfalfa leafcutter bees (*Megachile rotundata* F.) and in Kansas with honey bees (*Apis mellifera* L.) as pollinators. Both studies report significant gene flow from a genetic marker source to trap plots located at distances consistent with current AOSCA standards for commercial certified seed production. The objective of our study was to study gene flow under honey bee pollination under commercial seed production conditions in the San Joaquin of California.

Methods

The two year study was conducted in an alfalfa seed production area located in Fresno County, CA under normal commercial alfalfa seed production conditions. The study area contained seven alfalfa fields from which foraging honey bees were collected. The seven fields included one 318 acre RR transgenic herbicide-tolerant seed production field, four small 1.8 acre conventional alfalfa seed production fields (trap plots), one 240 acre conventional alfalfa seed production field, and one 55 acre conventional alfalfa seed production field (see Hagler et al., 2011a for details). The four small conventional fields were strategically planted in an equidistant linear fashion extending to the North along the west edge of the study between the RR field and the 240 acre conventional field starting at 165 ft. The 240 acre conventional field was located one mile to the North. Similar study areas were located within both the 240 and 55 acre commercial seed production fields. An additional 100 acre field located 5 miles from the RR source plot was also sampled at the time of commercial harvest. At harvest a total of 25 one-pound seed samples were taken in a systematic grid pattern from each study area. Each sample was evaluated f in a replicated field planting to detect the presence of the RR trait. Between 75,000 and 90,000 plants were tested for each trap plot resulting in a total of approximately 1.2 million seedlings tested in each of two years.

Results and Discussion

There was a logarithmic decrease in gene flow as distance from the source plot increased between 165 ft and 3 miles from the RR source field. Gene flow averaged 1.67% at 165 ft, 0.22 percent at 1 mile, 0.02% at 3 miles, and was not detectable at 5 miles. Under these commercial seed production conditions, gene flow was the same one mile from the source plot whether the intervening distance was bridged by the 1.8 acre trap plots or there was no intervening alfalfa. These results are in agreement with our earlier, smaller scale, study covering distances up to 2.5 miles from the marker source (Van Deynze et.al. , 2010). As might be expected in a bee pollinated cross fertilizing crop, gene flow is strongly associated with the frequency of foraging bees known to be from an apiary located adjacent to the RR source plot. These results are being utilized by seed certification agencies to develop isolation distances, and mapping and stewardship programs to be employed during the production of seed for markets that are sensitive to the presence of transgenic traits.

References

Association of Official Seed Certification Agencies. Alfalfa Seed Stewardship Program http://www.ccia.ucdavis.edu/identity_preserved/ASSP.htm

Brown, D.E. and E.T. Bingham. 1991. Selfing in alfalfa seed production fields. Proc. Western Alfalfa Improvement Conf. Boise, ID. 31 July-1 Aug. 1991. Western Alfalfa Improvement Conference.

California Crop Improvement Association. Alfalfa Isolation Management Map. http://alfalfa-gefree.cciamaps.ucdavis.edu/login.aspx?ReturnUrl=%2fdefault.aspx

California Crop Improvement Association. Certification standards alfalfa in California. http://ccia.ucdavis.edu/seed_cert/alfalfa_seedcert_standards.htm

Forage Genetics. 2001. Summary of gene flow studies 2000 and 2001. http://www.foragegenetics.com/GeneFlowSummary%201.ppt

Hagler, J.R., S. Mueller, L.R. Teuber, S.A. Machtley, and A. Van Deynze. 2011a. Foraging range of honey bees, *Apis mellifera*, in alfalfa seed production fields. Journal of Insect Science, *In Press.*

Hagler, J.R., S. Mueller, L.R Teuber, A. Van Deynze, and J. Martin. 2011b. A method for synchronous and distinct marking of honey bees, *Apis mellifera* originating from multiple apiary locations. Journal of Insect Science, *In Press.*

Knapp, E. E. and L. R. Teuber. 1993. Outcrossing rates of alfalfa populations differing in ease of floret tripping. Crop Science. 33:1181-1185.

St. Amand, P.C., Skinner, D.Z., and Peaden, R.N. 2000. Risk of alfalfa transgene dissemination and scale dependent effects. TAG 101: 107-114.

Teuber, L. R. , A. van Deynze, S. Mueller, K.L. Taggard, L. K. Gibbs, M. McCaslin, S. Fitzpartick, and G. Rogan. 2005. Gene flow. In alfalfa (Medicago sativa L.) when honey bees (Apis mellifera) are used as pollinators.). Proceedings of Alfalfa Seed Production Symposium. March 9 and 10, Five Points and Holtville, California. p. 11 – 15.

Van Deynze, A. E., S. Fitzpatrick, B. Hammon, M. H. McCaslin, D. H. Putnam, L. R. Teuber, D. J. Undersander. 2008. Gene Flow in Alfalfa: Biology, Mitigation, and Potential Impact on Production. Special Publicaiton No.28. Council for Agricultural Science and Technology. ISBN 978-1-887383-30-1

Challenges in organic alfalfa seed production in Southern California.

Ray Johnson, Top Notch Seed, Brawley, CA

My name is Ray Johnson. My wife and I are here from Holtville, California a small farming community in the Imperial Valley. We are located in the desert at the southern edge of the state between San Diego and Yuma, Arizona on the Mexico border.

I have been in agriculture since my youth, actively farming for 35 plus years. I've been involved in production, harvesting, processing, and shipping of many vegetable and field crops over that time. For the last 18 years Molly and I have been active partners in seed processing and production companies.

I have been asked to speak on the challenges of organic seed production in alfalfa. My focus is primarily on the desert region where we live for a few reasons. First because it is the area I know the most about. Secondly, the majority of the alfalfa seed production in California has moved from the San Joaquin Valley (Fresno area) to the Imperial Valley. Thirdly, our extreme weather and cropping systems make it easy to show the difficulties we face in gene flow.

I am not a certified organic grower, but my partner in the seed plant does operate a portion of his ranch under organic certification. We are certified organic processors and often partner financially with growers on organic production due to increased risk and cost.

We have been successful in organic alfalfa seed production as well as wheat, barley, and a few other crops. This success has been accomplished with timing, isolations, and more expensive irrigation methods among other things.

Organic acres and production in Imperial Valley are increasing every year, primarily in vegetable production. A good friend of mine says his demand for organic vegetable acres went up 30% last year. These acres are randomly mixed throughout the valley as I hope my photos will show.

One problem I found is our county crop and livestock report shows 6812 certified organic acres. The Ag commissioner's office knows that number is low, but if an application is submitted in another county that information might not reach our county office. Realistic estimates, I was given, ranged from 10,000 to possibly approaching 15,000 acres. These acres are spread through a valley of 400,000 plus acres.

Alfalfa is important to many of these organic acres, because it is the most common transition crop. A farmer can plant alfalfa and generate an income during the period necessary to qualify it as organic.

Imperial Valley has a very large forage hay and seed export industry. Both the hay and seed sales to other countries generate tens of millions each in income to the valley. Many of the receiving countries request GMO free products at this time. For this reason I have been urged to include conventional non GMO production in this discussion.

Most of the hay exports from our area are grasses, but more than 30% of our alfalfa hay production went off shore last year. This was of critical importance to us as a price support. Just as the domestic dairy industry demand starts to slow in the late spring, the exporters are able to start buying at prices that exceed dry cow values.

We have for many years been suppliers of high quality non dormant alfalfa seed to the world. For decades Imperial Valley growers and shippers have supplied both public and proprietary varieties to the U.S. and foreign buyers. University of California public varieties are the standard in many counties and countries while our contract production for various genetics companies continues to grow.

Another market we service is the horse industry. Horse feeding is a somewhat specialized market in terms of quality and what the buyer demands. Horses in general do not need the high feed value that a milking cow requires and can actually suffer health issues if fed dairy quality hay. The horse industry in the southwest has moved toward buying our summer hay from older stands with moderate grass weed content, or purchasing grass hay to mix with the alfalfa in their feeding schedule. Some of these consumers prefer that the hay be GMO free. We look at that as strictly a customer preference issue and to-date have no problem with supplying assurances on that issue.

Growers in the Imperial Valley have never had any particular problems meeting certification standards. We've always assumed that required isolation distances and dilution rates based on field size kept us true to type. However with the coming of GMO varieties and the ease of detecting gene flow from them we have had to address how to continue in the hay and seed production business.

The traditional practice in the valley has always been to plant hay fields at heavy seeding rates, farm them for a year or two or three, then when the stand has thinned out let a field or two go to seed. Most often the fields that want to make seed let you know. Soil types, insect pressures, micro climates, native pollinator presence, and other issues all have an influence. Many years I have called growers asking if they'll have any seed production that season and they answer, "No" not this year." Then a few weeks later they'll call me and say, "I sent the swathers out to cut field so and so and it's making seed like crazy. So I sent them back to the shop and ordered bees to pollinate"

Producers have always felt that this way of making their own seed took advantage of natural selection for their own area, cutting schedule, irrigation schedule, soils, and our summers. They say, "I want something that will survive down here." This method gives growers some control of their input costs, taking out the highs and lows of the seed market, plus they have absolute confidence in the quality and performance of the seed they will be planting the next year or two. This also gives a grower assurance of availability for a particular variety such as UC Cibola for planting on sandy ground; a variety developed in the 80's that still tops the trials in our area.

These practices and realities while being somewhat random in nature have never had much of an impact on a neighbor or the area, whether organic or conventional. Common practices that do impact your neighbor are common, such as cutting a hay field a certain direction, controlling insects and weeds, or others which have generally short term influence.

Conventional growers in the Imperial Valley are not anti GMO; however the majority feels that when GMO alfalfa is planted in the valley the gene flow will be unavoidable. This issue would have long lasting impact on organic and conventional production and marketing.

The strongest factor causing gene flow is our weather. Starting in the spring our temperatures range in the 90's and low 100's. Then by June and July we get 110-115 degrees with spikes up to 120, some years a day or two over 120. Some nights at midnight the bank thermometer in town still reads 100 or so. These conditions are normal during our seed production season. So, not only the seed alfalfa fields are under stress, but the hay fields are also trying to survive and naturally go into reproductive mode.

Much attention has been given to volunteer plants or planted spots just outside the cutting area which bloom all spring and summer, but we get entire 80 and 160 acre fields in full bloom for 10 or 15 days in close proximity to each other. The issue of controlling rogue plants around the ranch becomes minor in comparison. Many would say we need to cut the hay fields sooner. But, that is hard to justify when your concerns are preservation of the stand, maximizing yield, and profitability. With our temperature conditions an alfalfa field will not survive being cut at pre or early bloom multiple times. Also the reality is we sell alfalfa by the ton. We might get 10 or 15 dollars more per ton pre bloom, but the additional tonnage with maturity nearly always pays more, increases annual yield, reduces per ton harvest cost, and reduces replanting expenses.

Another issue making gene flow difficult to control in our region is the fact that farmers are somewhat randomly mixed throughout the valley. About 40% of the land in the valley is farmed by the owner according to the Imperial Irrigation District and even less than that on a full year round basis. The majority is tenant farmers and other arrangements. The result is many sizeable farmers are very spread out. So, it is very common for a field to have a different neighbor on 2, 3, or 4 sides. It is very rare for your neighbor to live close to those fields. He might live near you in town or he might live in a town 20 miles away. This does not lend itself to communication or planning on what is going to happen 50 or 60 feet away. Most farmers are friends, go to the same coffee shop, or have the same pest control advisor so communication does occur. But, sometimes neighbors are not friends and they may be competing for the same land, etc. I would not ask a friend to cut a field for my benefit.

An issue under this topic that has occurred lately is a land owner not renewing a lease on an alfalfa field and putting the field in the fallowing program with the irrigation district. The field is disced or worked to destroy the alfalfa. But by the rules must be cloddy to avoid blowing dust. Results are: the water goes to San Diego, the plants that survived bloom and make seed for a year, and nobody is watching. A friend harvested just such a field this season and took in 200 lbs per acre of seed after the field came out of the program.

A third challenge we experience is the movement of pollinators. We use both leafcutters and honey bees to pollinate a wide variety of crops many months of the year. There are many suppliers of those insects especially the honey bees. Some of the suppliers are local residents, but many are not. Often bee keepers from other areas will bring hives to the valley with no pollination agreements, but simply to make honey after the hills, west of us, dry up. It is very common for a seed producer to have good numbers of pollinators in a field and not know where they came from. I have had that in a field just north of town for 3 years in a row. I paid for leafcutter bees and did not think there was a need for honey bees, but they showed up in large numbers.

Putting honey bees on a field does not mean they will work that field. Many years ago a young farmer came to my office complaining his bees were flying to his neighbor's seed field. The next day the older gentleman neighbor came in saying, "Guess that young fella's mad at me." We went out to the field and sure enough, the bees were flying over one alfalfa field to work another. The older gentleman sent the younger a check for 6,000 dollars at the end of the season. Obviously he was a gentleman and saw what I'm saying was happening. In a similar situation a friend's bees were flying across the All American canal into Mexico doing the same thing. We could see two alfalfa fields in the direction the bees were flying, but did not drive into Mexico to seek compensation.

As a positive note Monsanto and the Imperial County Farm Bureau have collaborated on an agreement to not commercialize Roundup Ready alfalfa in the valley before July 31, 2014. Imperial County will be named in the Stewardship Agreements and in the Seed Dealer Agreements as a RR alfalfa free area.

Imperial County growers and shippers are very thankful for that cooperation by Monsanto. The many farm bureau meetings we had were well attended by local interested parties. We also were pleased to get attendance from alfalfa genetics companies from other states and counties as well as organic producers from local and adjoining areas.

Again, Imperial County Farm Bureau is not expressing an anti GMO statement nor are the growers and shippers. This agreement is seen as an opportunity to protect and not interrupt our current marketing methods and agreements. We are experiencing some increased interest in both organic and non GMO seed production from producers in other areas. Much of it is dormant and semi dormant varieties which are not normally grown in our climate.

It seems apparent that other areas have gene flow issues of their own and are looking for production options.

Many feel that the issue of coexistence is not in managing gene flow to prevent contamination. It is more an issue of how do organic and conventional producers deal with a readily identifiable unsolicited gene in their varieties or products? When will acceptances and or tolerances be established in various countries? Will I be able to produce my products and varieties in my back yard where I always have? How do we deal with it after we've got it, because we're all going to get it?

The Nature of Roadside Alfalfa (*Medicago sativa* L.) Populations and Implications for Genetically Engineered (GE) Trait Movement and Containment.

R. C. Van Acker[1]* and M.V. Bagavathiannan[2]
[1]University of Guelph, 50 Stone Rd, Guelph, ON, Canada, N1G 2W1. [2]University of Arkansas, Fayetteville, AR, 72704, USA.

Abstract

Genetically Engineered (GE) alfalfa has been deregulated in both the United States and Canada. As an outcrossing perennial, the ecology and biology of alfalfa is unique among deregulated GE crops. The potential for intra-specific GE trait movement may pose challenges for the coexistence of GE and non-GE alfalfa. The nature of roadside alfalfa populations is relevant to this consideration. There is an abundance of good evidence that alfalfa can readily establish and persist in roadside habitats without managed cultivation and can in this manner act as a reservoir for GE traits. For roadside populations there remains little information on seedbank dynamics, seedling recruitment, seed dispersal or the impact of novel traits on the performance of roadside alfalfa populations. Stewardship and co-existence programs for GE alfalfa need to consider the occurrence of feral populations in GE trait confinement and in co-existence plans and protocols. The degree to which feral alfalfa populations need to be managed and other stewardship practices implemented will depend on the nature of risk posed by the GE trait in question and the allowable AP threshold level. Total confinement of GE traits in alfalfa under normal field conditions is likely not practical in part because of the ubiquity and nature of roadside populations and the impracticality of their eradication. For traits that may not warrant total containment, establishing acceptable and practically attainable threshold levels will be essential for successful co-existence.

Introduction

The advent of genetic engineering has heightened awareness of the challenges and potential risks that can come with the development of GE traits in plants. Many risks associated with the release of GE crops are related to trait movement both from crop to wild or weedy species (inter-specific gene movement) and from crop to crop (intra-specific gene movement) (Marvier and Van Acker 2005). This is especially true for the movement of traits within and among farming systems and agricultural supply chains. Trait containment has become a challenge because of GE technology. With GE, there exists an extraordinary capability for the adventitious presence (AP) of novel traits, some of which can pose risks to human health and the environment. In addition, the principle of substantial equivalence of products derived from GE is not universally accepted, and the approach to and rate of deregulation is not universally consistent, leading to a chronic balkanized global market. The containment of GE traits has always been a challenge for regulated events (Marvier and Van Acker 2005). The inability to contain GE traits may jeopardize export markets for countries that rely on exports, including Canada and the United States, creating an unfavorable economic climate and uncertainties among exporters (Bagavathiannan et al. 2011c; Van Acker and Bagavathiannan 2011). In cases where traits can be contained, regulators can be much more permissive about which traits are allowed in crop plants; on the other hand, in cases where traits cannot be readily contained, technology developers and regulators need to be much more cautious about which traits are allowed, not only for widespread commercial release but also for cultivation in small, contained plots. In either case, traits that are regulated must be contained and there is zero tolerance for trait escape. Co-operative initiatives among international regulatory agencies could help establish protocols for effective containment of traits that warrant zero tolerance within specific production and supply chains (Bagavathiannan and Van Acker 2009b).

In North America, we have more than a decade of experience with commercial production of GE crops, providing a wealth of examples and evidence that bear on the consideration of GE trait movement and containment. This experience has provided two key lessons (Marvier and Van Acker 2005): 1) when GE crops are grown outside at a commercial scale, the movement of GE traits beyond their intended destinations is certain and the risk of escape increases with the scale of production. 2) full retraction of escaped GE traits is unlikely.

These points support the need for caution and serious consideration where there is a hope or expectation of co-existence and commercial segregation, especially for situations where a GE trait is regulated. Trait movement is especially complex within large agricultural supply chains that involve many actors and living elements across an active landscape (Van Acker et al. 2007). Traits may persist and move among living populations of plants, including feral and volunteer plants, and among latent populations in seed that may exist in a myriad of places within the production and supply chain. In any case, the role of volunteer and feral populations and latent seed populations in trait persistence and movement can be substantive. As such, this needs to be well recognized and understood for trait risk assessment purposes and for the consideration of commercial co-existence or segregation schemes.

Alfalfa (*Medicago sativa* L.) is the most important forage species in North America and GE alfalfa has been deregulated in both the United States and Canada. As an outcrossing perennial, the ecology and biology of alfalfa is unique among deregulated GE crops. The potential for intra-specific GE trait movement in alfalfa has been studied and considered (Van Deynze et al. 2008), but one area where there has been limited study is in the nature of roadside populations and the role they can play in GE trait movement.

The nature of roadside alfalfa

In studies conducted in Manitoba, Canada, we characterized the nature of a range of roadside alfalfa populations by studying these populations both *in situ* and in controlled experiments. The life-history characteristics of alfalfa suggest that it is a candidate species for high gene flow and ferality potential (Bagavathiannan and Van Acker 2008). Alfalfa can escape cultivation and establish self-sustaining populations in unmanaged habitats. Alfalfa cultivars are typically selected for persistence under grass mixtures, and the traits that favour their adaptation as a cultivated crop also favour their persistence in roadsides. In particular, the ability to fix nitrogen, presence of deep tap roots, drought and cold tolerance, perenniality, high genetic diversity, and fast regrowth potential are the key traits that favour successful establishment in competitive environments (Bagavathiannan and Van Acker 2009a). Alfalfa populations are commonly observed in roadsides and unmanaged habitats, particularly in alfalfa growing regions (Fitzpatrick et al. 2003; Kendrick et al. 2005; Prosperi et al. 2006). We found feral roadside populations to be commonplace in southern Manitoba (Bagavathiannan et al. 2011b), and also learned that these populations were not genetically distinct from typical commercial alfalfa cultivars (Bagavathiannan et al. 2010b), indicating that these were typical escapes from cultivation. Such escape could happen during farming activities (i.e. planting, harvesting, transport operations, etc.) or through intentional planting in roadsides, which is not uncommon in N. America.

The demography of roadside alfalfa suggests that it is capable of establishing self-perpetuating populations in roadside habitats, with key facilitating elements being persistent seedbanks, successful seedling recruitment, and adult reproductive success (Bagavathiannan et al. 2010a). Alfalfa grows well despite the limited nutrient levels in roadside habitats (Drenovsky et al. 2008). Mowing affects the reproductive success of roadside alfalfa, but roadsides are typically not completely mowed (Bagavathiannan 2009). Roadside alfalfa populations are very similar in growth performance to cultivated alfalfa, except for fecundity where cultivated alfalfa in a typical production field produces more than 3 times as much seed as an average roadside alfalfa plant. This may be related to delayed maturity caused by competition (McGraw et al. 2008) leading to low mature seed production prior to mowing or due to insects such as lygus bugs (*Lygus spp*.) (Soroka 1991). Nevertheless, the levels of seed production we found in roadside alfalfa populations appeared to be sufficient to perpetuate these populations (Bagavathiannan et al. 2010a). There was a difference between seed production levels and seedbank densities, which may be related to high seed predation levels for protein rich alfalfa seeds. In western Canada, average hard seed content for cultivated alfalfa seed ranged from 14 to 37% (Fairey and Lefkovitch 1991). Alfalfa seed had a good ability to overwinter in roadside conditions (overwintering mortality ranged from only 14 to 24%; Bagavathiannan et al. 2010a). There were high levels of winter mortality for seedlings (> 80% after 2 years), especially for seedlings emerging near mother plants as would be expected (Jennings and Nelson 1991). Nevertheless, we observed a considerable number of alfalfa seedlings to survive for two years (Bagavathiannan et al. 2010a) and evidence from the literature suggests that seedlings that survive for two years will most likely continue to survive (Rumbaugh 1982). In establishment experiments we found that alfalfa recruited very well in a typical grass sward and recruitment levels in the first year ranged between 0.5% and 9.7%. Plant density for fall seed dispersal was 82% lower than for spring dispersal. Low plant density was compensated for over time by increased numbers of shoots and racemes plant^{-1}, which increased seed output. Herbicide (2,4-D) application effectively controlled all emerged alfalfa plants but some dispersed seeds remained dormant forming a seedbank which drove recruitment in subsequent years (Bagavathiannan et al. 2011a).

Phenotypic characterization provided evidence that roadside alfalfa populations were experiencing selection pressure for adaptive traits including winter survivability, rhizome production and prostrate growth habit, traits that favor persistence in unmanaged habitats (Bagavathiannan et al. 2010b). We also noted the occurrence of high (62 to 85%) levels of outcrossing in feral alfalfa populations.

Recently, we developed a stage-structured matrix population model for roadside feral alfalfa populations that we had been studying in southern Manitoba (Bagavathiannan et al. 2011d, in review). The model accounted for the existence of density-dependence and recruitment subsidy in feral populations and was parameterized using field data and existing literature. The model output suggested that typical roadside feral alfalfa populations will persist, and the likelihood for extinction is minimal, especially under current roadside management regimes practiced in southern Manitoba. Long-term persistence of alfalfa populations in pastures and rangelands has been reported by several authors (Kilcher and Heinrichs 1965; Pearse 1965; Rumbaugh and Pedersen 1979). Feral alfalfa populations experience density-dependent regulation of population growth, leading to equilibrium population densities. Our observations of roadside alfalfa populations suggest that seedling establishment is affected by density-dependence due to auto-allelopathy and limited dispersal abilities. Seed immigration generally increased equilibrium densities, but was not an absolute requirement for sustaining populations. In a timely-mowing scenario, the survival of the population was dependent on seed immigration, but only if local seed production was completely prevented. It appears that the dynamics of feral alfalfa is seed limited in populations where mowing prevents seed production and recruitment limited where the roadside is not mowed. In a timely-mowing scenario, feral alfalfa populations steadily decline if seed production is assumed to be completely prevented. Population extinction however, is slow because seedbank exhaustion for a typical roadside seedbank could take up to 7 years.

Managing feral alfalfa for trait containment

Current isolation distance requirement for certified alfalfa seed production in Canada is 50 meters and for foundation seed it is 200 meters (for fields exceeding 5 acres) or 300 meters (for fields that are 5 acres or less) (CSGA 2003). These isolation distances are designed to achieve variety purity (within limits) but not necessarily genetic purity (or the prevention of GE trait entry). As such, and given the evidence of long distance pollen mediated gene flow (PMGF) in alfalfa (St. Amand et al. 2000; Fitzpatrick et al. 2003), isolation distances may need to be revisited. However, the appropriate isolation distance may be dictated by the nature of the GE trait and the level of AP allowed.

Hay fields need to be managed properly and cut regularly before flowering. In a survey in southern Manitoba, we found flowering plants in many hay fields with flowering synchrony occurring between feral populations and hay fields in 1/3 of cases (Bagavathiannan 2009).

Producers who wish to maintain GE-free alfalfa will need to make conscientious efforts to do so and need to better understand the routes and mechanisms of GE trait movement (Van Acker et al. 2007). The identification and management of feral alfalfa population will be required, particularly in scenarios where AP thresholds are very low.

Some GE traits may facilitate the persistence of feral populations including traits favoring adaptation including drought and salt tolerance and pest and disease resistance. In addition, traits that confer herbicide resistance may be a concern if the associated herbicide is broad spectrum (e.g. glyphosate) and used to control weeds along roadways, right-a-ways and volunteer GE alfalfa in subsequent crops.

Conclusion

There is an abundance of good evidence that alfalfa can readily establish and persist in roadside habitats without managed cultivation and can in this manner act as a reservoir for GE traits. There remains little information on the seedbank dynamics of roadside alfalfa populations and estimates of persistence are not robust. In addition, there has been limited study of alfalfa seedling recruitment *in situ*, especially away from mother plants, and these estimates of recruitment are also not robust. Alfalfa seed dispersal is poorly understood yet its nature has substantive implications for population establishment, growth and sustenance. Studies on the nature of feral alfalfa remain rare and our results, although broad in scope, are limited in terms of the geography and genotypes represented. In addition, the likelihood of persistence, spread and invasion of feral populations can be influenced by introduced traits (Claessen et al. 2005 a,b) and this has not been well studied in alfalfa

Stewardship and co-existence programs for GE alfalfa need to consider the occurrence of feral populations in GE trait confinement and in co-existence plans and protocols. The degree to which feral alfalfa populations need to be managed and other stewardship practices should be implemented will depend on the nature of risk posed by the GE trait in question and the resultant allowable AP threshold level in GE-sensitive production systems. Total confinement (and achieving zero-tolerance) of GE traits under normal field conditions is likely not practical, and alfalfa is not a good candidate crop for traits that require absolute containment. For traits that may not warrant total containment, establishing acceptable and practically attainable threshold levels are essential for successful co-existence of GE and GE-sensitive crop production systems in agricultural landscapes. Further, enactment and enforcement of appropriate regulations is vital.

References

Bagavathiannan, M. V. and R. C. Van Acker. 2008. Crop ferality: implications for novel trait confinement. Agric. Ecosyst. Environ. 127:1-6.

Bagavathiannan, M. V. 2009. Feral Nature of Alfalfa (*Medicago sativa* L.): Implications for Novel Trait Confinement. Ph.D. Thesis. University of Manitoba. Pp. 227.

Bagavathiannan, M. V. and R. C. Van Acker. 2009a. The biology and ecology of feral alfalfa (*Medicago sativa* L.) and its implications for novel trait confinement in North America. Crit. Rev. Plant Sci. 28:69-87.

Bagavathiannan, M. V. and R. C. Van Acker. 2009b. Transgenes and national boundaries – the need for international regulation. Environ. Biosafety Res. 8:141-148.

Bagavathiannan, M. V., R. H. Gulden, G. S. Begg, and R. C. Van Acker. 2010a. The demography of feral alfalfa (*Medicago sativa* L.) populations occurring in roadside habitats in Southern Manitoba, Canada: implications for novel trait confinement. Environ. Sci. Pollut. Res. 17:1448-1459.

Bagavathiannan, M. V., B. Julier, P. Barre, R. H. Gulden, and R. C. Van Acker. 2010b. Genetic diversity of feral alfalfa (*Medicago sativa* L.) populations occurring in Manitoba, Canada and comparison with alfalfa cultivars: an analysis using SSR markers and phenotypic traits. Euphytica 171:419-432.

Bagavathiannan, M. V., R. H. Gulden, and R. C. Van Acker. 2011a. The ability of alfalfa (*Medicago sativa*) to establish in a seminatural habitat under different seed dispersal times and disturbance. Weed Sci. 59:314-320.

Bagavathiannan, M. V., R. H. Gulden, and R. C. Van Acker. 2011b. Occurrence of feral alfalfa (*Medicago sativa* L.) populations along roadside habitats in southern Manitoba, Canada and their role in intraspecific novel trait movement. Trans. Res. 20:397-407.

Bagavathiannan M.V., A. Spok, and R. C. Van Acker. 2011c. Commercialization of perennial GE crops: looming challenges for regulatory frameworks. J. Agric. Environ. Ethics 24:227-242.

Bagavathiannan, M. V., G. S. Begg, R. H. Gulden, and R. C. Van Acker. 2011d. The population dynamics of feral *Medicago sativa* L. (alfalfa) and management implications. Ecol. Appl. 2X: (in review).

Claessen, D., C. A. Gilligan, P. J. W. Lutman, and F. Van den Bosch. 2005a. Which traits promote persistence of feral GM crops? Part 1: implications of environmental stochasticity. OIKOS 110:20-29.

Claessen, D., C. A. Gilligan, and F. Van den Bosch. 2005b. Which traits promote persistence of feral GM crops? Part 2: Implications of metapopulation structure. OIKOS 110:30-42.

CSGA (Canadian Seed Growers' Association). 2003. Regulations and procedures for pedigreed seed production. Circular 6-94. Section 7. Available at: http://www. seedgrowers. ca/cropcertification/index.asp?lang=e; accessed 19 August, 2011.

Drenovsky, R. E., C. E. Martin, M. R. Falasco, and J. J. James. 2008. Variation in resource acquisition and utilization traits between native and invasive perennial forbs. Am. J. Bot. 95:681-687.

Fairey, D. T. and L. P. Lefkovitch. 1991. Hard-seed content of alfalfa grown in Canada. Can. J. Plant Sci. 71:437-444.

Fitzpatrick, S., P. Reisen, and M. McCaslin. 2003. Pollen-mediated gene flow in alfalfa: a three year summary of field research. In: Proceedings of 2003 Central Alfalfa Improvement Conference. Available at: http:www.naaic.org/meetings/central 2003; accessed 11 August, 2011.

Jennings, J. A. and C. J. Nelson. 1991. Reseeding old alfalfa stands. In: Proceedings of the 1991 American Forage and Grassland Council Conference, Columbia, MO, USA, pp 162-165.

Kendrick, D., T. Pester, M. Horak, G. Rogan, and T. Nickson. 2005. Biogeographic survey of feral alfalfa populations in the U.S. during 2001 and 2002 as a component of an ecological risk assessment of Roundup Ready Alfalfa®. In: Proceedings of North Central Weed Science Society Meeting, UT, USA.

Kilcher, M. R. and D. H. Heinrichs. 1965. Persistence of alfalfa in mixture with grasses in a semiarid region. Can. J. Plant Sci. 46:163-167.

Marvier, M. and R. C. Van Acker. 2005. Can crop transgenes be kept on a leash? Front. Ecol. Environ. 3: 93-100.

McGraw, R. I., W. T. Stamps, J. H. Houx, and M. J. Linit. 2008. Yield, maturation, and forage quality of alfalfa in a black walnut alley-cropping practice. Agroforest. Syst. 74:155-161.

Pearse, C. K. 1965. Range study tour in the Soviet Union. J. Range Manage. 18:305-310.

Prosperi, J. M., E. Jenczewski, M. Angevain, and J. Ronfort. 2006. Morphologic and agronomic diversity of wild genetic resources of *Medicago sativa* L. collected in Spain. Gen. Res. Crop Evol. 53: 843-856.

Rumbaugh, M. D. 1982. Reseeding by eight alfalfa populations in a semiarid pasture. J. Range. Manage. 35:84-86.

Rumbaugh, M. D. and M. W. Pedersen. 1979. Survival of alfalfa in five semiarid range seedings. J. Range. Manage. 32:48-51.

Soroka, J. J. 1991. Insect pests of legume and grass crops in western Canada. Agriculture and Agri-Food Canada Publication no. 1435, 39pp.

St. Amand, P., D. Skenner, and R. Peaden. 2000. Risk of alfalfa transgene dissemination and scale-dependent effects. Theor. Appl. Genet. 101:107-114.

Van Acker, R.C. and M. V. Bagavathiannan. 2011. Volunteer and feral crop plants and latent seed populations play an important role in novel trait containment. Pages XXX-XXX. in H. Beckie and L. Hall eds. Topics in Canadian Weed Science, Volume 9. Sainte-Anne-de Bellevue, Québec: Canadian Weed Science Society – Société canadienne de Malherbologie (in press).

Van Acker, R. C., N. McLean, and R. C. Martin. 2007. Development of quality assurance protocols to prevent GM-contamination of organic crops. Pages 466-489 in Handbook of Organic Food Safety and Quality, J. Cooper, U. Niggli and C. Leifert (Eds), CRC Press, Boca Raton, FL,USA.

Van Deynze, A., S. Fitzpatrick, B. Hammon, M. H. McCaslin, D. H. Putnam, L. R. Teuber, and D. J. Undersander. 2008. Gene Flow in Alfalfa: Biology, Mitigation and Potential Impact on Production. Council for Agricultural Science and Technology (CAST) Publication Series, Ames, IA, USA.

An overview of male sterility strategies for transgene biocontainment

Hong S. Moon[1] and C. Neal Stewart, Jr.[1]*
[1] University of Tennessee, 2431 Joe Johnson Dr. Knoxville, TN 37996
* Presenting author
nealstewart@utk.edu

Abstract

Male sterility has been one of the most extensively studied strategies for biological transgene containment. Genetically engineered complete male sterility could be an effective approach to prevent pollen-mediated transgene escape from genetically engineered crops. Genic and cytoplasmic male sterility biotechnological tools are the primary technologies that have been developed. However, complete male sterility strategies could bring some negative ecological consequences to pollinators and pollen-feeding insects due to lack of pollen. A novel selective male sterility strategy by ablating transgenic pollen has been developed and being characterized. Current data of selective male sterility suggest highly efficient transgenic pollen ablation. This strategy might be an ideal approach for safe and reliable biological transgene containment to control transgene escape through pollen dispersal. In addition to male sterility technologies, another pollen biocontainment method uses site-specific recombination or another excision technology to remove transgenic DNA from pollen. Taken together, most male sterility technologies are generally in their infancy and there are advantages and disadvantages to each. It is clear, however, that for many crops and transgene types, biocontainment will be required for biosafety and regulatory purposes.

Introduction

Transgene containment strategies are currently considered as essential components for genetically engineered (GE) crop cultivation in several cases, such as when recombinant pharmaceutical proteins are produced and for many perennial plants. Biological containment strategies have been considered more efficient and reliable methods compared to physical strategies including spatial distance and fences to contain unwanted transgene escape from GE crops. Male sterility has been most studied component of biocontainment. In the reproductive cycle of higher plants, fertile pollen is required for successful double-fertilization via transmission of the sperm cells to the ovule. Complete containment of pollen-mediated transgene escape and introgression could be achieved by prevention of pollen formation or production of infertile pollen, which would obviate the potential for long-distance transgene dispersal via wind or insect pollinators. In addition to male sterility, it is also generally acknowledged that female sterility, especially selective female sterility will be important for transgene biocontainment. Both male a female biocontainment strategies will be briefly introduced here with an emphasis on novel technologies that are being developed.

Genic male sterility

Disrupting pollen development using genetic engineering has been suggested for containing transgene escape and introgression (Daniell, 2002; Feil et al., 2003). Multiple methods have been used to prevent pollen formation or decrease pollen fertility via genic or cytoplasmic male sterility. Many male sterile plants have been genetically engineered using constructs that disrupt the tapetum, a layer of cells found within the pollen sac, essential for pollen development (reviewed in Daniell, 2002). The first transgenic male sterile plant was generated by genetic engineering of tobacco plants with the chimaeric ribonuclease gene (Mariani et al., 1990). Most genic male sterile plants have been achieved by using tapetum-specific promoters to drive the expression of toxic bacterial genes (e.g. *Barnase* from *Bacillus amyloliquefaciens*, diphtheria toxin A), resulting in no pollen formation (Hird et al., 1993; Koltunow et al., 1990; Lee et al., 2003). Since then, several genetic engineering efforts have been demonstrated to develop other genic male sterility approaches and applications in plants. These include using cytotoxic *barnase* gene expression in pollen or anthers of poplar (*Populus*) trees and *Kalanchoe blossfeldiana* (Wei et al., 2007; García-Sogo et al., 2010).

Cytoplasmic male sterility

Another approach to generate male sterile plants is via cytoplasmic male sterility (CMS) (Chase, 2006). One approach is CMS that blocks the production of functional pollen using mutations in the plant mitochondrial genome (Hanson & Bentolila, 2004). CMS plants are thought to have utility for limiting transgene escape via pollen dispersal (Feil et al., 2003). Genetically engineered CMS has been developed for biological transgene containment as well (Ruiz & Daniell, 2005). This was achieved by genetic engineering of tobacco (*Nicotiana tabacum* L.) chloroplast genome with the *phaA* gene coding for ß–ketothiolase, which is known to confer CMS (Ruiz & Daniell, 2005). A potential drawback of using CMS as a biological transgene containment tool is the potential for transmission of the transgene from the cytoplasm to the nucleus. Transmission of paternally-inherited plastids and mitochondria in crosses involving parents with an alien cytoplasm occurs at low frequency in many plant species (Svab & Maliga, 2007). Also the loss of fertility in a CMS breeding plant population could eventually be restored under natural conditions (Schnable & Wise, 1996).

Advantages and disadvantages of genic sterility and CMS

Both genic and cytoplasmic male sterility approaches should be considered the best and most reliable biological transgene containment mechanisms for the containment of transgenic pollen; thereby short circuiting possible hybridization, introgression and unwanted transgene persistence. However, these approaches might not be considered to be ecologically friendly approaches. Since these complete male sterility strategies inhibit development of anther or pollen, the lack of pollen could create negative impacts on pollen-feeding insect food chain (Mlynárová et al., 2006).

Transgenic pollen ablation has been demonstrated by expression of the diphtheria toxin gene under the control of the LAT52 pollen-specific and putative pectin esterase promoter in tobacco (Twell, 1995; Uk et al., 1998). However, using these cytotoxic genes that have been used in most male sterility studies might be a concern of potential toxicity to non-targeted organisms or cells. This potential cytotoxicity would not be a problem if a gene coding for non-cytotoxic protein were used for male sterility.

GM gene-deletor: clipping transgenes out of pollen cells

Transgene introgression could be effectively suppressed post-hybridization with the removal of the transgene from the pollen. Pollen-specific transgene excision using site-specific recombinases, such as Cre or FLP (Luo et al., 2007; Moon et al., 2010), is one method to create transgene-free pollen carrying only a non-coding recombination site. Efficient microspore-specific transgene excision has been demonstrated in tobacco (*Nicotiana tabacum* cv. Petit Havana SR1) using Cre recombinase directed by a microspore-specific NTM19 promoter

(Mlynárová et al., 2006). Other recombinases, including ParA and PhiC31, that have been shown to excise transgenes in plants, have the potential to be used for pollen-specific transgene excision (Thomson et al., 2009; Kempe et al., 2010). One novel resolvase, CinHI was adapted for transgene pollen excision in plants (Moon et al. 2011). If perfected, pollen would not carry any functional transgenes after transgene excision occurred. One large drawback of any gene deletor system is that it requires several extra kb of DNA and multiple levels of new intellectual property at a time where most regulators and companies value minimal DNA insertion and simple licensing deals.

A novel selective male sterility system

Cell death has been shown to be conferred by the induced expression of the EcoRI restriction endonuclease in yeast, *Saccharomyces cerevisiae* (Barnes & Rine, 1985). Induced expression of the EcoRI restriction endonuclease was lethal to transformed *Escherichia coli* and suppressed the growth of the cells (Gholizadeh et al., 2010). We have begun a project to overexpress the EcoRI restriction endonuclease in pollen cells for the purpose selectively disabling the genome of transgenic pollen. A pollen-specific Lat5X promoter from tobacco was placed upstream of an EcoRI-GFP translational fusion construct and introduced into tobacco. Several independent transgenic events showed 100% transgenic pollen ablation based on currently available data. Hemizygous transgenic events containing single copy of the EcoRI gene would have 50% of ablated transgenic pollen and 50% normal non-transgenic pollen. Thus, only transgenic pollen will be destroyed. There might be concerns regarding potential negative effects of restriction endonuclease on insect pollinators. If transgenic pollen were completely ablated, insect pollinators should not be exposed to restriction endonuclease protein.

Conclusion

It is doubtful that there will emerge one perfect male –sterility biocontainment system for all transgenic crop species and transgene systems. None is optimal today for any system, although many are useful. Since regulations are becoming more stringent at the same time that developers are prognosticating more diverse crops and traits, it is inevitable that male sterility will become vital to assuring biosafety of pollen spread in crops in a regulatory and commercial framework with higher frequency.

References

Barnes G, Rine J (1985) Regulated expression of endonuclease EcoRI in *Saccharomyces cerevisiae*: Nuclear entry and biological consequences. *Proc. Natl. Acad. Sci. USA* 82:1354-1358

Chase CD (2006) Genetically engineered cytoplasmic male sterility. *Trends Plant Sci.* 11:4-9

Daniell H (2002) Molecular strategies for gene containment in transgenic crops. *Nat. Biotechnol* 20:581-586

Feil B, Weigartner U, Stamp P (2003) Controlling the release of pollen from genetically modified maize and increasing its grain yield by growing mixtures of male-sterile and male-fertile plants. *Euphytica* 130:163-165

García-Sogo B, Pineda B, Castelblanque L, Anton T, Medina M, Roque E, Torresi C, Beltran JP, Moreno V, Canas LA (2010) Efficient transformation of *Kalanchoe blossfeldiana* and production of male-sterile plants by engineered anther ablation. *Plant Cell Rep.* 29:61-77

Gholizadeh A, Faizi MH, Baghban Kohnehrouz B (2010) Induced expression of EcoRI endonuclease as an active maltose binding fusion protein in *Escherichia coli*. *Microbiology* 79:167-172

Hanson MR, Bentolila S (2004) Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell* 16:S154-S169

Hird DL, Worrall D, Hodge R, Smartt S, Paul W, Scott R (1993) The anther-specific protein encoded by the *Brassica napus* and *Arabidopsis thaliana* A6 gene displays similarity to beta-1,3-glucanases. *Plant J.* 4:1023-1033

Kempe K, Rubtsova M, Berger C, Kumlehn J, Schollmeier C, Gills M (2010) Transgene excision from wheat chromosomes by phage phiC31 integrase. *Plant Mol. Biol.* 72:673-687

Koltunow AM, Truettner J, Cox KH, Wallroth M, Goldberg RB (1990) Different temporal and spatial gene expression patterns occur during anther development. *Plant Cell* 2: 1201-1224

Lee Y-H, Chung K-H, Kim H-U, Jin Y-M, Kim H-I, Park B-S (2003) Induction of male sterile cabbage using a tapetum-specific promoter from *Brassica campestris* L. ssp. *pekinensis*. *Plant Cell Rep.* 22:268-273

Luo K, Duan H, Zhao D, Zheng X, Deng W, Chen Y, Stewart CN Jr., McAvoy R, Jiang X, Wu Y, He A, Pei Y, Li Y (2007) GM-gene-deletor': fused *loxP-FRT* recognition sequences dramatically improve the efficiency of FLP or CRE recombinase on transgene excision from pollen and seed of tobacco plants. *Plant Biotechnol. J.* 5:263-274

Mariani C, De Beuckeleer M, Truettner J, Leemans J, Goldberg RB (1990) Induction of male sterility in plants by a chimaeric ribonuclease gene. *Nature* 347:737-741

Mlynárová L, Conner AJ, Nap JP (2006) Directed microspore-specific recombination of transgenic alleles to prevent pollen-mediated transmission of transgenes. *Plant Biotechnol J.* 4:445-452

Moon HS, Li Y, Stewart CN, Jr. (2010) Keeping the genie in the bottle: transgene biocontainment by excision in pollen. *Trends Biotechnol.* 28:3-8

Moon, H.S., L.L. Abercrombie, S. Eda, R. Blanvillain, J.G. Thomson, D.W. Ow, C.N. Stewart, Jr. 2011. Transgene excision in pollen using a codon optimized serine resolvase CinH-*RS2* site-specific recombination system. Plant Molecular Biology 75:621-631.

Ruiz ON, Daniell H (2005) Engineering cytoplasmic male sterility via the chloroplast genome by expression of ß-ketothiolase. *Plant Physiol.* 138:1232-1246

Schnable PS, Wise RP (1998) The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci.* 3:175-180

Svab, Z. Maliga, P. (2007) Exceptional transmission of plastids and mitochondria from the transplastomic pollen parent and its impact on transgene containment. *Proc. Natl. Acad. Sci. USA* 104:7003-7008

Thompson, C. J. *et al*. (2003) Model-based analysis of the likelihood of gene introgression from genetically modified crops into wild relatives. Ecol. Model. 162:199-209

Twell D (1995) Diphtheria toxin-mediated cell ablation in developing pollen: vegetative cell ablation blocks generative cell migration. *Protoplasma* 187:144-154

Uk KH, Seok PS, Park YD, Jin YM (1998) Pollen ablation of transgenic tobacco plants by expression of the diphtheria toxin A-chain gene under the control of a putative pectin esterase promoter from Chinese cabbage. *Mol. Cells* 30:310-317

Wei H, Meilan R, Brunner AM, Skinner JS, Ma C, Gandhi HT, Strauss SH (2007) Field trial detects incomplete *barstar* attenuation of vegetative cytotoxicity in *Populus* trees containing a poplar *LEAFY* promoter::*barnase* sterility transgene. *Mol. Breed.* 19:69-85

Cross-Incompatibility Systems in *Zea mays*

Matthew M. S. Evans*[1]
[1]Carnegie Institution for Science, Department of Plant Biology, 260 Panama St., Stanford, CA 94305

Abstract

Pollination is key to seed production in the most important cereal crops and consequently is key in the production of most of the world's food supply. In wind-pollinated species, which lack mate selection based on species-specific pollinator identity, the interaction between the pollen grain/tube and the pistil is the point of selection for an appropriate mate by the female. Within *Zea mays*, natural hybrids between maize and teosinte, the wild progenitor of maize, are relatively uncommon, even when growing in intimate association with each other and flowering simultaneously. Three loci, *teosinte crossing barrier1* (*tcb1)*, *gametophyte factor1* (*ga1*), and *ga2*, contribute to reproductive isolation between *Zea mays* populations by conferring unilateral cross-incompatibility between the pistils of some plants and the pollen of others. Three naturally occurring haplotypes exist for each locus: the neutral haplotypes (*tcb1*, *ga1*, or *ga2*) which accept all maize and teosinte pollen; the strong haplotypes (*Tcb1-s*, *Ga1-s*, or *Ga2-s*), which contains the pistil function preventing pollination by the neutral haplotype and also contains the pollen function allowing fertilization of (*Tcb1*, *Ga1*, or *Ga2*)*–s* females; and the male only haplotypes (*Tcb1-m*, *Ga1-m*, or *Ga2-m*), which contains the male function allowing fertilization of females of the appropriate strong haplotype but lacks the female function and so can be fertilized by pollen of the neutral haplotype. The *Tcb1-s* locus contains genetically distinct male and female factors that can be separated by recombination. Additionally, the barriers to cross-pollination in all three systems occur within the silk after pollen germination and are manifested as a reduction in pollen tube length in incompatible crosses.

Introduction

Interaction between pollen and pistil governs reproductive compatibility in flowering plants. Accordingly, pollen-pistil incompatibility establishes the boundaries of hybridization by preventing close inbreeding in some taxa as well as preventing wide, dysgenic outcrossing. These interactions are governed by pistil expressed and pollen expressed genes. The self-incompatibility (SI) systems of Solanaceae, Brassicaceae, and Papaveraceae are the best characterized systems of such interactions and have been shown to consist of two tightly linked but distinct genes that encode the pistil and pollen functions (TAKAYAMA and ISOGAI 2005).

In wind-pollinated species, which lack mate selection based on pollinator identity, the interaction between the pollen grain/tube and the pistil is the point of selection for an appropriate mate by the female. Typically, angiosperm families with abiotic pollinators like wind have less species diversity than those with biotic pollinators, but the grasses (Poaceae/Gramineae) have an higher species diversity than typical for such taxa (DODD *et al.* 1999).

Zea mays subspecies including domesticated maize provide an excellent opportunity to study these phenomena. Wild Mexican teosinte pollen can fertilize cultivated maize when hand crossed, but, for many strains of teosinte, fertilization of teosinte by maize often fails due to physiological incompatibility between teosinte silks and maize pollen. Specifically, maize pollen fertilizes plants of certain annual teosinte populations poorly if at all. Incompatibility is especially strong in ssp. *mexicana* teosinte populations that grow in intimate association with maize as a weed, and that flower in synchrony with it (BALTAZAR *et al.* 2005; ELLSTRAND *et al.* 2007). This cross-incompatibility (CI) is hypothesized to provide the reproductive isolation necessary to maintain them as separate populations (KERMICLE *et al.* 2006). Because these CI barriers are unilateral it is possible to transfer them from teosinte to maize using recurrent hand-pollinations to standard maize lines to facilitate their study.

Three different genetic systems conferring unilateral cross-incompatibility have been discovered in *Zea mays* populations. These are: *teosinte crossing barrier1* (*tcb1*), *gametophyte factor1* (*ga1*), and *ga2* (EVANS and KERMICLE 2001; KERMICLE 2006; KERMICLE and EVANS 2010; KERMICLE *et al.* 2006; NELSON 1994). The genetically dominant (but less frequent in domesticated maize) haplotypes *Tcb1-strong* (*Tcb1-s*), *Ga1-strong* (*Ga1-s*), and *Ga2-strong* (*Ga2-s*) confer barriers to cross-pollination by the recessive haplotypes *tcb1*, *ga1*, and *ga2*. The recessive haplotypes which accept all types of pollen are found in all (*tcb1*) or most (*ga1* and *ga2*) domesticated maize varieties. There also exist naturally occurring haplotypes that have the pollen function but not the pistil function, *Tcb1-male* (*Tcb1-m*), *Ga1-m*, and *Ga2-m* (*i.e.* they can pollinate -*s* haplotype females but do not discriminate against pollen of the recessive, *tcb1*, *ga1*, or *ga2* haplotypes). These cross-incompatibility systems are analogous to self-incompatibility, particularly gametophytic self-incompatibility, in that they are characterized by a sporophytically encoded female function that rejects unwanted pollen and a gametophytically encoded male function that allows for circumventing this pistil barrier. Because the ability of the pollen to function is determined gametophytically it is possible for a *Tcb1-s*/*tcb1* heterozygote to reject half of its own pollen — those grains carrying the *tcb1* allele — in a self-pollination. In these cases of cross-incompatibility it has not yet been reported whether the male and female functions are provided by separate genes like SI or by a single gene expressed in both tissues.

Many steps have to be performed by the pollen grain after landing on the stigma to achieve fertilization and seed production (reviewed in (EDLUND *et al.* 2004; SANCHEZ *et al.* 2004)). When a maize pollen grain lands on the silk, it typically adheres to one of the stigmatic hairs along the length of the silk and hydrates and germinates within the first 5-15 minutes after contact (BARNABAS 1985; BARNABAS and FRIDVALSZKY 1984; BEDINGER and FOWLER 2009). The pollen germinates and extends a tube via polarized tip growth of the vegetative cell. After germination, the pollen tube must navigate the tissues of the pistil to reach the embryo sac and achieve fertilization. The maize silk can be up to 40cm long, and multiple pollen grains typically land on each silk with only one grain achieving fertilization of the embryo sac. This presents a strong selection for rapid pollen tube growth in maize with growth rates as high as 1cm/hour

(BARNABAS and FRIDVALSZKY 1984), and this growth must occur through the existing cell walls of the transmitting tract. Consequently, in addition to the cellular functions required for tip growth of the pollen tube, pollen tubes need additional functions to interact with and penetrate the female tissues for growth *in vivo*. The pollen grain produces, or carries in the pollen coat from tapetal secretions, some of the proteins that are necessary for this process, and indeed pollen coat proteins have been shown to have potent cell wall loosening activity (COSGROVE *et al.* 1997; SUEN and HUANG 2007; VALDIVIA *et al.* 2007).

In *Ga1-s* conditioned cross-incompatibility, incompatible pollen tubes enter the transmitting tract and arrest growth without reaching the ovule, in contrast to inter-specific crosses in which the pollen tubes fail to target the transmitting tract correctly (HOUSE and NELSON 1958; LAUSSER *et al.* 2010). Our data indicate that a similar situation is the case for *Tcb1-s* and *Ga2-s*. Additionally, using fine mapping experiments, we have been able to show that the male and female functions of *Tcb1-s* can be separated from one another by recombination.

Results and Discussion

Enhancement of *Tcb1-s*

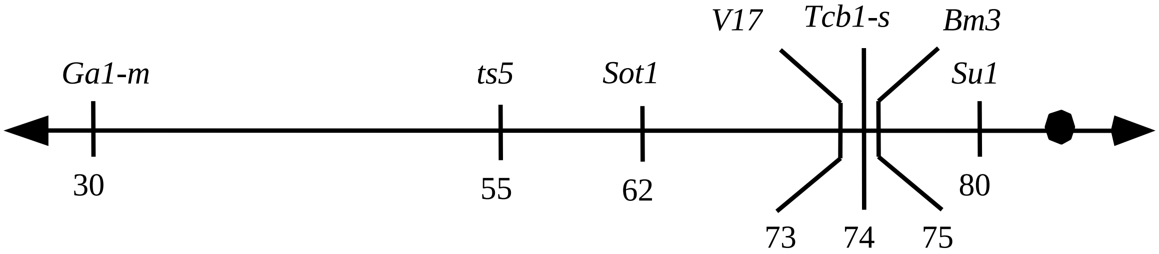


Figure 1. The organization of the short arm of chromosome 4 carrying the Teosinte Incompatibility Complex haplotype from a teosinte accession of *Zea mays* ssp. *mexicana* containing *Ga1-m Sot1* and *Tcb1-s*. The approximate positions of classical maize genes *tassel seed5* (*ts5*), *viriscent17* (*v17*), *brown midrib3* (*bm3*), and *sugary1* (*su1*) are shown.

Because the *tcb1* and *sugary1* loci are linked to one another, in self-pollinations of *Tcb1-s +/tcb1 su1* heterozygotes, the *tcb1 su1* pollen grains are selected against by the *Tcb1-s* silks. In circumstances with strong *Tcb1-s* action, the only *su1* kernels from these self-crosses (~3% of the total) are from recombinant *Tcb1-s su1* pollen grains fertilizing *su1* embryo sacs. This is the case for plants carrying the full Teosinte Incompatibility Complex (TIC) encompassing the short arm of chromosome 4 that includes *Tcb1-s*, *Ga1-m,* and intervening loci from the original teosinte collection of *Tcb1-s* (EVANS and KERMICLE 2001). In some crosses involving *Tcb1-s* the barrier to *tcb1* pollen is incomplete. In these crosses seed set from the incompatible pollen is almost exclusively towards the tip of the ear, and the percentage of *su1* kernels in *Tcb1-s +/tcb1* *su1* self-crosses is variable, ranging from 3% to as high as 14% (still significantly below 25%). Using recombinants between *tcb1* and *ga1* on the short arm of chromosome 4 it was determined that a second locus about 12 cMorgan distal to *tcb1* had an allele from teosinte that stabilized the *Tcb1-s* barrier (Figure 1). Replacing the teosinte allele with the maize allele of this locus by recombination leads to the barrier of variable strength, usually weaker than the full TIC. This locus has been termed *Stabilizer of Tcb1-s* (*Sot1*).

Pollen tube growth in incompatible crosses

Figure 2. (A) Unpollinated maize ear showing long silks originating at the base of the ear (bottom) and shorter silks originating at the tip. (B) *Ga1*-s ear pollinated by *ga1* pollen showing scattered seed set clustered in the distal half of the ear. (C) Ears pollinated by a mix of *Ga2-s*, *r1-r* pollen (conditioning yellow kernels) and *ga2*, *R1-self color* pollen (conditioning purple kernels). The top ear is *ga2 tcb1 ga1* accepting all pollen and revealing the ratio of pollen in the mix (*i.e.* a large excess of *ga2* pollen). The bottom ear is *Ga2-s* revealing the selection against *ga2* pollen. Note that successful incompatible fertilization of *Ga2-s* by *ga2* is biased toward the tip of the ear.

Our crosses with *Tcb1-s* (as well as *Ga1-s* and *Ga2-s*) females in conditions with incomplete rejection of *tcb1 ga1 ga2* pollen revealed that when seed set occurred it was at the tips of the ears (Figure 2). This biased seed set is seen both when the incompatible pollen is used exclusively (Figure 2B) or in competition assays when the incompatible pollen is provided in great excess (Figure 2C). These are the flowers that have the shortest styles in the maize ear (Figure 2A), demonstrating that incomplete rejection of pollen allowed fertilization of flowers with short styles but not long styles. This result in turn suggested that rejection of cross-incompatible pollen occurred via a restriction of pollen tube growth within the style rather than via a barrier to gamete fusion at the ovule or a barrier to pollen germination on the surface of the pistil.

Recombinational separation of the pollen and pistil functions of *Tcb1-s*

To determine the number of genetic components constituting the *Tcb1-s* haplotype and assist their identification, we have conducted a fine mapping study of the region by selecting for recombinants between the flanking markers *viriscent17* (*v17*) and *brown midrib3* (*bm3*) in crosses of + *Tcb1-s* +/*v17 tcb1 bm3* heterozygous males onto *v17 tcb1 bm3* females. Recombinants of both types, *V17*$^{+}$ *bm3* and *v17 Bm3*$^{+}$, were scored in reciprocal crosses between the recombinants and a *Tcb1-s* tester to test for pollen and pistil *Tcb1-s* function. Out of a population of 16,451 individuals we isolated one recombinant carrying only the male function (*i.e.* it could fertilize *Tcb1-s* silks but accepted *tcb1* pollen) and one recombinant carrying only the female function (*i.e.* it could not fertilize *Tcb1-s* silks but rejected *tcb1* pollen). Not only did this data indicate that the two components are discrete genetic entities (albeit very tightly linked), but it also demonstrated that the male factor is distal to the female factor (Figure 3).

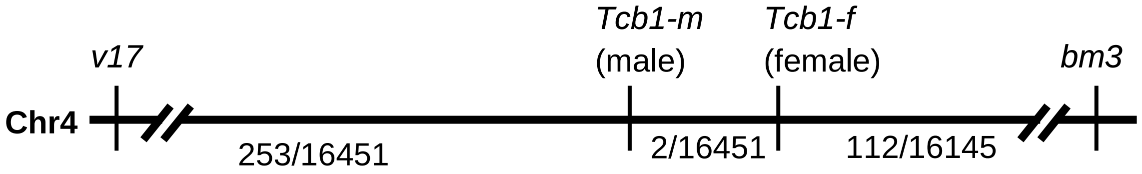


Figure 3. Genetic map of the *Tcb1-s* haplotype showing the relative order of the male and female genes making up *Tcb1-s* and the genetic distance between them.

Crosses of *Tcb1-s* silks with pollen functionally heterozygous for *Tcb1-s/tcb1* demonstrate full functionality of the heterozygous pollen on *Tcb1-s* females. The *Tcb1-s* male gene therefore appears to provide function to the pollen that *tcb1* lacks allowing tube growth rather than *tcb1* providing a function absent in *Tcb1-s* that leads to pollen rejection. This suggests a model for *Tcb1-male* function in promoting growth in a *Tcb1-s* silk environment, while the female factor perhaps causes a biochemical difference in the style that *tcb1* pollen is ineffective in penetrating. This model that also fits with the *Ga1-s* and *Ga2-s* systems based on parallel sets of experiments.

References

BALTAZAR, B. M., J. J. SÁNCHEZ GONZÁLEZ, L. DE LA CRUZ-LARIOS and S. J. B., 2005 Pollination between maize and teosinte: an important determinant of gene flow in Mexico. Theor Appl Genet **110:** 519-526.

BARNABAS, B., 1985 Effect of water loss on germination ability of maize (*Zea mays* L.) pollen. Annals of Botany **55:** 201-204.

BARNABAS, B., and L. FRIDVALSZKY, 1984 Adhesion and germination of differently treated maize pollen grains on the stigma. Acta Botanica Hungarica **30:** 329-332.

BEDINGER, P. A., and J. E. FOWLER, 2009 The maize male gametophyte, pp. 57-77 in *Handbook of Maize: Its Biology*, edited by J. L. BENNETZEN and S. C. HAKE. Springer, New York.

COSGROVE, D. J., P. BEDINGER and D. M. DURACHKO, 1997 Group I allergens of grass pollen as cell wall-loosening agents. Proc Natl Acad Sci U S A **94:** 6559-6564.

DODD, M. E., J. SILVERTOWN and M. W. CHASE, 1999 Phylogenetic Analysis of Trait Evolution and Species Diversity Variation among Angiosperm Families. Evolution **53:** 732-744.

EDLUND, A. F., R. SWANSON and D. PREUSS, 2004 Pollen and stigma structure and function: the role of diversity in pollination. Plant Cell **16 Suppl:** S84-97.

ELLSTRAND, N. C., L. C. GARNER, S. HEGDE, R. GUADAGNUOLO and L. BLANCAS, 2007 Spontaneous hybridization between maize and teosinte. J Hered **98:** 183-187.

EVANS, M. M. S., and J. L. KERMICLE, 2001 Teosinte crossing barrier1, a locus governing hybridization of teosinte with maize. Theoretical and Applied Genetics **103:** 259-265.

HOUSE, L. R., and O. E. NELSON, 1958 Tracer study of pollen-tube growth in cross-sterile maize. J. Heredity **49:** 18-21.

KERMICLE, J. L., 2006 A selfish gene governing pollen-pistil compatibility confers reproductive isolation between maize relatives. Genetics **172:** 499-506.

KERMICLE, J. L., and M. M. EVANS, 2010 The Zea mays sexual compatibility gene ga2: naturally occurring alleles, their distribution, and role in reproductive isolation. J Hered **101:** 737-749.

KERMICLE, J. L., S. TABA and M. M. S. EVANS, 2006 The gametophyte-1 locus and reproductive isolation among Zea mays subspecies. Maydica **51:** 219-225.

LAUSSER, A., I. KLIWER, K. O. SRILUNCHANG and T. DRESSELHAUS, 2010 Sporophytic control of pollen tube growth and guidance in maize. J Exp Bot **61:** 673-682.

NELSON, O. E., 1994 The gametophyte factors in maize, pp. 406-503 in *The Maize Handbook*, edited by M. FREELING and V. WALBOT. Springer-Verlag, New York.

SANCHEZ, A. M., M. BOSCH, M. BOTS, J. NIEUWLAND, R. FERON *et al.*, 2004 Pistil factors controlling pollination. Plant Cell **16 Suppl:** S98-106.

SUEN, D. F., and A. H. HUANG, 2007 Maize pollen coat xylanase facilitates pollen tube penetration into silk during sexual reproduction. J Biol Chem **282:** 625-636.

TAKAYAMA, S., and A. ISOGAI, 2005 Self-incompatibility in plants. Annu Rev Plant Biol **56:** 467-489.

VALDIVIA, E. R., Y. WU, L. C. LI, D. J. COSGROVE and A. G. STEPHENSON, 2007 A group-1 grass pollen allergen influences the outcome of pollen competition in maize. PLoS ONE **2:** e154.

EPIGENETIC SILENCING OF FLORAL GENES

Zhongchi Liu[1]* and Dandan Li[1]
[1] University of Maryland, Dept. of Cell Biology and Molecular Genetics, Univ. of Maryland, College Park, MD 20742

Abstract

Introns, occupying higher percentages of the plant genome than protein-coding exons, are not static blocks of intervening sequencings. They play regulatory roles through a variety of mechanisms. We showed that over-expressing the first and longest intron of the *Arabidopsis thaliana CAULIFLOWER* (*CAL*) gene silenced the endogenous *CAL* gene expression, which correlated with the simultaneous production of intron-derived siRNA and DNA methylation at the *CAL* locus. The silenced phenotype is inherited at a high efficiency for several generations even in the absence of the original intron-containing transgene. The mechanism, if further understood, could be utilized to silence genes of choice and improve crop traits, while at the same time eliminating the triggering transgene. Thus, intron-triggered gene silencing can be potentially developed as a new tool in "epigenetic engineering", mitigating concerns of transgene flow.

Introduction and results

Small interfering RNA (siRNA) regulates gene expression through post-transcriptional and transcriptional mechanisms (Bonnet et al., 2006; Chen, 2009). In the "post-transcriptional gene silencing (PTGS)", siRNAs, usually 21-22 nucleotides in length, guide the RISC (RNA-Induced Silencing Complex) to degrade homologous mRNA in the cell. In contrast, "transcriptional gene silencing (TGS)" occurs, when the siRNA guides an ARGONAUTE 4 (AGO4)-bound complex to facilitate de novo DNA methylation in the Cytosine at CG, CHG, and CHH. This process is coiled "RNA-dependent DNA Methylation (RdDM)".

To silence a gene for biotechnological applications, transgenic plants were often engineered to produce double stranded RNAs (dsRNAs) for a specific gene. The dsRNAs were then cleaved by DCL1 (DICER1) into 21-22 nt siRNAs that act via PTGS to degrade the target mRNA. In almost all of the cases, the silenced phenotype is inherited as long as the dsRNA-producing transgene is inherited. In contrast to PTGS, there isn't an effective method to specifically induce TGS. Our recent work showed that it is possible to induce TGS of a gene by over-expressing a gene's intron. While we do not yet know why some over-expressed introns could induce TGS, the mechanism, if further understood, could be utilized to silence genes of choice and improve crop traits.

CAL was originally identified by a mutation that enhanced mutants of *ap1-1* (Bowman et al., 1993). It was later found to encode a MADS box protein highly similar to *AP1* (Kempin et al., 1995). *cal* single mutants are wild type in phenotype, while *ap1* single mutants convert floral meristems partially into an inflorescence meristem. *cal-1* mutation dramatically enhances *ap1-1* so that the *ap1-1; cal-1* double mutation fully converts floral meristems into inflorescence meristems, resulting in the "cauliflower" phenotype.

We found that over-expressing the first intron of an *Arabidopsis* gene *CAULIFLOWER (CAL)* could induce silencing of endogenous *CAL*, resulting in a characteristic cauliflower-like (*cal*-like) floral phenotype in the *apetala1 (ap1)* mutant background. Further, this intron-triggered silencing correlated with the production of intron-derived siRNA and DNA methylation at the *CAL* locus. Most importantly, the silenced phenotype was inherited at a high efficiency for several generations even after the original intron-containing transgene was segregated away. Further analysis of how the intron-triggered silencing is transmitted is ongoing. We propose that the ability to trigger TGS holds the promise of engineering new traits that are maintained for generations even when the initial transgene is eliminated. This "epigenetic engineering" may help mitigate concerns of transgene flow**.**

Reference

Bonnet, E., Van de Peer, Y. and Rouze, P. (2006). The small RNA world of plants. *New Phytol* 171, 451-68.
Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E. M. and Smyth, D. R. (1993). Control of flower development in Arabidopsis thaliana by APETALA1 and interacting genes. *Development* 119, 721-743.
Chen, X. (2009). Small RNAs and their roles in plant development. *Annu Rev Cell Dev Biol* 25, 21-44.
Kempin, S. A., Savidge, B. and Yanofsky, M. F. (1995). Molecular basis of the cauliflower phenotype in Arabidopsis. *Science* 267, 522-5.

Male Sterility in Hybrid Systems

Marc C. Albertsen

Agricultural Biotechnology, Pioneer Hi-Bred Int. Inc., Johnston, IA, 50131, USA

Agricultural gene flow occurs whenever a crop is grown in the vicinity of a sexually compatible species, regardless of whether the crop is transgenic. Gene flow from agricultural crops has been occuring ever since the first humans selected plants that were of benefit to their food security. Agricultural gene flow often is considered only from the perspective of pollen from the crop pollinating a compatible species, with the possibility of pollen from the compatible species pollinating the crop plant overlooked. Progeny from this latter type of gene flow also has the possibility of ending up as volunteers in the field the following season. The likelihood of this scenario is much reduced, but should be kept in mind as we consider the entire gene flow spectrum.

Reproductive biology research has a long history at Pioneer Hi-Bred, not only with the first cloning of a male fertility gene in maize, but also with the identification of many genetic components that are required for controlling the expression of male fertility genes. As a result, we have developed several pollination control systems, some based upon dominant gene mechanisms and some based on recessive gene mechanisms. One of these is a novel genetic process to increase a nuclear genetic male sterile inbred that then can be utilized in hybrid seed production, designated as SPT (Seed Production Technology). This unique process utilizes a proprietary, genetically modified maintainer line that enables propagation of the male sterile parent line that is uniquely not transgenic for the SPT process. Therefore, pure populations of non-transgenic, male-sterile female parent lines are produced. SPT utilizes a naturally occurring recessive mutation of a sporophytic gene required for male fertility to create female parent lines that are male sterile when the mutant allele is made homozygous. Full male fertility is restored in hybrid plants upon pollination of the male-sterile female parent plants with pollen from any male parent that is, by default, carrying the wild-type allele of the mutant. The SPT process offers a reliable, cost-effective method to propagate pure populations of homozygous recessive male-steriles during female parent seed increase that are non-transgenic for the SPT process.

Title: **Mitigating Gene Flow from Seed - an Energy Crop Perspective**

Authors: Michael F. Portereiko[1]*, Steven Bobzin[1], Roger Pennell[1], Richard Flavell[1].

[1]Ceres, Inc., 1535 Rancho Conejo Blvd, Thousand Oaks, CA 91320.

Abstract:

Herbaceous Dedicated Energy Crops (DECs) will play a significant role in the long-term future of biofuels and biopower. Switchgrass, *Miscanthus*, and high biomass and sweet sorghums are likely to figure prominently in the portfolio of DECs deployed in the US and other countries. The use of transgenes to improve performance, yield, and sustainability in order to improve biomass sources is a near certainty. It could be that transgene containment may be useful for the management, stewardship, and deregulation of these transgenic crops. Agronomic practices and current biotechnology approaches that restrict gene flow through pollen represent a first step toward mitigating transgene flow to the environment. If strict sterility were the goal then restriction of gene flow through both the pollen and the seed, i.e. total sterility, may prove to be useful for the release of certain transgenically improved varieties. Seed sterility has been introduced in the past with less than favorable reception, especially in the context of crops that are grown for commercial food and feed production. In the context of DECs it is useful therefore to re-evaluate the role of genetic confinement strategies that eliminate seed as part of the larger picture of biotechnology stewardship.

Introduction:

The United States' desire for energy security, a carbon-neutral economy, and a rebuilding of the Nation's rural sector, have all led to the understanding that domestically grown biomass will play a significant role in the generation of bioenergy and bioproducts over the coming decades (Energy Independence and Security Act of 2007 (EISA); Perlack and Stokes 2011). Initially, some of the biomass will come from waste streams and wood chips, however, it is clear that Dedicated Energy Crops (DECs) with high yields and low input requirements will transform the energy sector. Among these DECs are switchgrass (*Panicum virgatum*), *Miscanthus,* and sorghum (*Sorghum bicolor*), all of which are C4 grasses that can feed into the bioenergy and bioproduct markets. Sweet sorghum, for example, which produces a high level of soluble sugars in the stalk, is useful for processing in 1st generation biofuel plants that convert simple sugars to ethanol. These plants could be used immediately utilizing the existing infrastructure of sugar cane processing facilities. High biomass sorghums, as well as switchgrass and *Miscanthus,* produce copious amounts of complex cellulosic material that is suitable for biopower production or usable in 2nd generation biofuel plants that convert cellulose to liquid fuels.

All of these crops have many desirable characteristics, including the ability to grow on limited inputs and are high yielding. However, it is arguable that the only way to attain both bioenergy output and sustainability goals will be to incorporate transgenics into the bioenergy crop portfolio (Gressel 2008; Sticklen 2009). 1st generation transgenic traits (i.e. herbicide tolerance and insect resistance) have helped to increase grain yield in crops such as corn and soy since their introduction in the 1990s. Since both weeds and insect damage place significant downward pressure on biomass output and farmers are generally familiar with these technologies with an estimated 66.8 million hectares planted in biotech crops bearing these traits in the U.S. in 2010 (James 2010), it would not be surprising if these 1st generation traits were adopted by farmers in large-scale transgenic DEC plantings. A new wave of biotechnological traits is beginning to crest. These types of traits include modifications that enable plants to grow with fewer inputs, including, for example, less nitrogen fertilizer and less water, features that are already prevalent features of DECs, as well as traits that enable the production of pharmaceuticals or other co-products. Nitrogen and water use efficiency traits are likely to prove valuable from both an economic and environmental standpoint. Economically, reductions in fertilizer use and irrigation reduce the direct costs of farming and increase the amount of available acreage for the crops. Environmentally, these traits can reduce the impact on local hydrology and the contamination of streams and rivers. The first expected use of such traits is anticipated in the coming years.

The most prevalent avenues of gene flow from field-based transgenic species into the environment are 1) pollen flow from transgenic plants to either sexually compatible native species or non-transgenic cultivars of the same crop and 2) the dispersal of transgenic seed away from the field plots; transgenic canola (rapeseed), for example, has been identified alongside the rail systems in Canada (Yoshimura, Beckie et al. 2006), and it is thought that this has occurred primarily through the leakage of canola seeds from rail cars as they travel to their shipping and distribution points. Methods of limiting such dispersal include physical, genetic, and biotechnological strategies. Physical strategies include pollen containment (e.g. physical barriers, large distances between crops, and/or the planting of crops where no native species exist), actions to limit seed dispersal (e.g. field maintenance to watch for volunteer crops, sealed seed transport containers, and barriers to limit animal access to the crops), and environmental approaches, such as managing flowering time. However, without careful safeguards in place, the establishment of transgenic plants far from the original agronomic intent can occur (Reichman, Watrud et al. 2006).

Both genetic and biotechnological approaches exist to minimize gene flow from the male. One genetic approach is the use of cytoplasmic male sterility (CMS) systems. CMS is a condition under non-nuclear genetic control that results in the inability of the male gametophyte (pollen) to contribute to sexual reproduction. Engineered male sterility through the use of the barnase/barstar system has also been developed and has been demonstrated in tobacco, cauliflower, cotton, tomato, corn, lettuce, and others. Both of these male-sterility approaches aim to reduce the transmission of transgenes from the male in breeding programs and during hybrid production where the source of the pollen needs to be managed. Chloroplast transformation, which has been demonstrated in numerous species, including soybean, cotton, tobacco, potato, and tomato, among others, (Clarke, Daniell et al. 2011) aims to minimize gene flow through the male by taking advantage of the mostly female-specific transmission of chloroplast DNA. At the moment, this technology has not proven itself in monocots, which include rice, wheat, corn, sorghum, and the energy grasses. Mechanisms to minimize seed production in DECs include daylength-sensitive varieties and genetic use restriction technologies. Daylength-sensitive varieties can be grown in geographic regions that either severely delay or eliminate completely the cues that trigger the transition to reproductive growth. These plants produce considerably more biomass and available resources, such as sugars, for the biofuels stream since they do not convert energy into seed product. Genetic use restriction technology, or GURT, is the name given to proposed methods for restricting the use of genetically modified plants by causing second generation seeds to be sterile (Hills, Hall et al. 2007).

Each of these methods for confinement comes with its own caveats and potential pitfalls. For example, male-sterility technology alone may prove of limited value for DECs. For a plant like switchgrass, when grown in its native habitat, which includes much of the United States east of the Rocky Mountains, pollen from wild relatives could enable seed production that can escape into the environment and produce transgenic volunteers. In addition, male-sterile sorghums can become infected by ergot, a fungus that infects unfertilized ovules and leads to potentially serious economic losses to the harvestable yield. One caveat to the daylength-sensitivity approach for genetic confinement is that it is environmentally regulated, and thus significant variability is possible from year to year and from one geographic region to another. One type of GURT initially developed by the US Department of Agriculture and Delta and Pine Land Co (US Patent 5,723,765) is designed to prevent germination of seed harvested from transgenic plants. This so-called “terminator” technology has not been commercialized anywhere in the world due to opposition from farmers, indigenous peoples, civil society and some governments, including a legal ban in both Brazil and India. The biggest concern raised against the technology is that it might lead to dependence for poor farmers who have a tradition of saving seed from each year’s crop to use in the following year. This same argument could potentially be used against all technologies that limit on-plant seed production. The significance of this concern is questionable, in light of the mounting use of hybrid seed in crop production, the use of company/customer agreements that legally prevent farmers from saving seed, and the fact that alternative non-transgenic seed stocks are available.

The most effective genetic confinement systems will be ones that can eliminate gene flow from both pollen and seed. For DECs, additional research needs to be used to address expanding the existing technology to cover seed and to invest in new research on alternative methods of mitigating gene flow through seeds. Reconsideration should be given to genetic confinement systems that reduce the risk of gene flow from seeds for several reasons, including the fact that the elimination of seed should contribute to substantial gains in energy-yield per acre, the relative ease of which energy crops can be propagated by seed transmission, and the fact that switchgrass and sorghum pollinators are abundant in the wild. The whole topic of risk assessment of transgenic crops is one that will inevitably generate controversy; regulators should retain a science-based risk assessment process with the goals of harmonizing standards internationally (Wolt 2009).

References

Clarke, J., H. Daniell, et al. (2011). "Chloroplast biotechnology, genomics and evolution: current status, challenges and future directions." Plant Mol Biol **76**: 207-9.

Gressel, J. (2008). "Transgenics are imperative for biofuel crops." Plant Science **174**: 246-263.

Hills, M., L. Hall, et al. (2007). "Genetic use restriction technology (GURTs). Strategies to impede transgene movement." Trends Plant Sci **12**: 177-183.

James, C. (2010). Brief 42: Global Status of Commercialized Biotech/GM Crops: 2010. ISAAA Brief. Ithaca, NY, The International Service for the Acquisition of Agri-biotech Applications (ISAAA). **42**.

Perlack, R. and B. Stokes (2011). U.S Billion-Ton Update: Biomass Suplly for a Bioenergy and Bioproducts Industry. U. S. D. o. Energy, Oak Ridge National Laboratory, Oak Ridge, TN**:** 227p.

Reichman, J., L. Watrud, et al. (2006). "Establishment of transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) in nonagronomic habitats." Molecular Ecology **15**(13): 4243-4255.

Sticklen, M. (2009). "The biofuels agenda shall not wait for miracles!" Biofuels, Bioproducts and Biorefining **3**: 419-421.

Wolt, J. (2009). "Advancing environmental risk assessment for transgenic biofeedstock crops." Biotechnol Biofuels **2**: 27.

Yoshimura, Y., H. Beckie, et al. (2006). "Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada." Environ. Biosafety Res. **5**: 67-75.

Chloroplast Genetic Engineering to Facilitate Maternal Inheritance of Transgenes and Confer Cytoplasmic Male Sterility

Henry Daniell
Department of Molecular Biology and Microbiology, University of Central Florida, College of Medicine, Orlando FL 32816-2364

Abstract

One among the major concerns in genetically modified (GM) crops is the possibility of the foreign gene (transgene) escaping and outcrossing into other non-GM crops or wild/weedy relatives. While transgenes integrated into the nuclear genome are carried via pollen and could result in outcross, chloroplast genomes are maternally inherited in most crops. Therefore, genetic modification approaches like chloroplast transformation (insertion of transgenes into the chloroplast genome) offers an attractive solution for controlling gene flow between genetically modified crops and their wild relatives or organically grown non-GM crops. Another attractive option would be to genetically engineer cytoplasmic male sterility (CMS) via the chloroplast genome. The concept of chloroplast transformation was first established in tobacco in the past decades and has now been extended to major crop species including cotton, soybean and monocots (cereals). Field tests with USDA-APHIS approval have been conducted in the United States for crops producing vaccines or human therapeutic proteins (that may not be permitted for engineering via the nuclear genome). Filed tests for soybean engineered for enhanced agronomic traits (herbicide/insect resistance) via the chloroplast genome are in progress. In addition to conferring transgene containment, there are several other advantages of chloroplast genetic engineering, including very high levels of foreign gene expression conferring exceptionally high levels of biotic or abiotic stress tolerance or production of foreign proteins up to 75% of the total leaf protein.

In plants exhibiting *Lycopersicon*-type maternal inheritance, chloroplasts are shunted to the vegetative cell during the 1st pollen mitotic division during pollen formation; none are found in the generative cell from which the sperm cells arise. The paternal chloroplasts shunted to the vegetative cell are generally destroyed when the pollen tube (derived from the vegetative cell) penetrates the synergid cell prior to fertilization. There are several other known mechanisms for elimination of chloroplast DNA present in pollen. Therefore, integration of transgenes into the chloroplast genome is an important approach to accomplish both transgene biocontainment and high levels of transgene expression, without the possibilities for gene silencing or position effects [1, 2]. Maternal inheritance of genetically modified chloroplast genomes and the absence of any reproductive structures when foreign proteins expressed in leaves are harvested, offer efficient transgene containment via pollen or seeds and facilitates their safe production in the field [3]. Two recent studies confirm efficient control of maternal inheritance of transgenes in transplastomic tobacco [4]. Ruf et al [5] set up a stringent selection system for paternal transmission by using male sterile maternal parents and transplastomic pollen donors conferring plastid specific antibiotic resistance and green fluorescence for visual screening. This selection system identified six among 2.1 million seedlings screened (frequency of $2.86 \text{ X } 10\text{-}^{6}$) that showed paternal transmission of transgenes and the authors concluded that plastid transformation provides an effective tool to increase biosafety of GM crops. In addition, transplastomic plants producing human therapeutic proteins have been already tested in the field after obtaining USDA-APHIS approval [6].

Integration of transgenes into the chloroplast genomes will greatly limit the transmission of transgenes via pollen and therefore prevent outcross to other crops or relatives. However, if transgene products (including vaccines, biopharmaceuticals, industrial enzymes, polymers, etc.) are harvested from leaves before the appearance of any reproductive structures, efficient transgene containment via pollen or seeds is possible. The major technical challenge to this containment strategy is to get the transgene into every chloroplast (homoplasmy) in each cell. However, only three rounds of selection on regeneration media is typically required to achieve homoplasmy in tissues regenerated via organogenesis, like tobacco or lettuce. Southern blots and PCR are used to detect wild type (untransformed) copies of the chloroplast genome and homoplasmic lines are identified and propagated. Since chloroplasts are prokaryotic compartments, they lack the silencing machinery found within the cytoplasm of eukaryotic cells and facilitate multi-gene engineering in a single transformation step because chloroplast genome naturally contains many operons. Each plant cell contains 50-100 chloroplasts and each chloroplast contains ~100 copies of its genome. Therefore, it is possible to introduce up to 10,000 copies of the transgene per cell. The highest levels of expression reported in the published literature for engineering agronomic traits used chloroplast transformation to express the *Bacillus thuringiensis* insecticidal protein, up to 46% of total leaf protein [7]. Similarly a human therapeutic protein - proinsulin was expressed up to 72% of the total leaf protein [8] using this concept. Surprisingly, transplastomic plants maintained normal growth and reproduction despite such hyper-expression of foreign proteins. Compartmentalization of toxic foreign proteins within chloroplasts minimizes or eliminate pleiotropic effects of the transgene. In some cases, expression of biomass hydrolysis enzymes (β-glucosidase) doubled the biomass by releasing active hormones from inactive conjugates stored within chloroplasts [9].

Transgenes have been stably integrated and expressed via the tobacco chloroplast genome to confer important agronomic traits including herbicide, insect, and disease resistance, drought and salt tolerance, cytoplasmic male sterility or phytoremediation [1, 2]. Chloroplast genomes of several crop species including cotton, soybean, carrot, eggplant, sugarbeet, cauliflower, cabbage, oilseed rape, poplar, potato, tomato, tobacco, lettuce and other crops have been transformed [1,2]. Twenty four vaccine antigens against 16 different diseases and twelve biopharmaceuticals including insulin and interferon have been expressed in tobacco chloroplasts and many are fully functional [10]. Complete chloroplast genome sequences of more than thirty crop species have been determined recently [11], facilitating rapid advancement in this field. Chloroplast transformation in cereal crops was first reported in rice [12] and more recently in wheat [13]. Aforementioned points on gene containments and chloroplast transformation have been recently reviewed by this author [14].

The first engineered cytoplasmic male sterility system in plants was accomplished by expression of β-kethiolase by stable integration of the phaA gene via the chloroplast genome [15]. Prior attempts to express the phaA gene in transgenic plants were unsuccessful. The phaA gene was efficiently transcribed in all tissue types including leaves, flowers and anthers. Coomassie-stained gel and western blots confirmed hyper-expression of β-ketothiolase in leaves and anthers, with proportionately high levels of enzyme activity. The transgenic lines were normal except for the male sterile phenotype, lacking pollen. Scanning electron microscopy revealed a collapsed morphology of the pollen grains. Floral developmental studies revealed that transgenic lines showed an accelerated pattern of anther development, affecting their maturation and resulted in aberrant tissue patterns. Abnormal thickening of the outer wall, enlarged endothecium and vacuolation affected pollen grains and resulted in the irregular shape or collapsed phenotype. Engineered cytoplasmic male sterility was restored by increasing photoperiod which enhanced ACCase activity and diverted Acetyl CoA from β-kethiolase, thereby reversing male sterility. This reversible male sterility method offers yet another tool for transgene containment and provides an expedient mechanism for F1 hybrid seed production. Aforementioned points on cytoplasmic male sterility were recently reviewed by this author [14].

References

1) Verma D, Daniell H: Chloroplast vector systems for biotechnology applications. Plant Physiology 145:1129-1145 (2007).

2) Clarke JL, Daniell H: Plastid biotechnology for crop production: present status and future perspectives. Plant Molecular Biology 76: 211-220 (2011)

3) Daniell H: Molecular strategies for gene containment in transgenic crops. *Nat. Biotechnol.* 20, 581-586 (2002).

4) Daniell H: Transgene containment by maternal inheritance: effective or elusive? *Proc. Natl. Acad. Sci. U S A* 2007, 104**:**6879-6880.

5) Ruf S, Karcher D, Bock R: Determining the transgene containment level provided by chloroplast transformation. *Proc. Natl. Acad. Sci. USA* 104, 6998-7002 (2007).

6) Arlen PA, Falconer R, Cherukumilli S, Cole A, Cole AM, Oishi KK, Daniell H: Field production and functional evaluation of chloroplast-derived interferon-alpha2b. *Plant Biotechnol J* 5**:** 511-25 (2007).

7) B. DeCosa, W. Moar, S. B. Lee, M. Miller and H. Daniell Hyper-expression of the Bt Cry2Aa2 operon in chloroplasts leads to formation of insecticidal crystals, Nature Biotechnology, 19:71-74 (2001).

8) Ruhlman T, Verma D, Samson N, Daniell H: Role of heterologous elements in transgene integration and expression. Plant Physiology, 152: 2088-2104 (2010).

9) Jin S, Kanagaraj A, Verma D, Lange T, Daniell H: Breakthrough Technologies: Release of hormones from conjugates: Chloroplast expression of B-glucosidase results in elevated phytohormone levels with significant increase in biomass and protection from aphids and whiteflies conferred by sucrose esters. Plant Physiology 155: 222-235

10) Daniell H, Singh ND, Mason H, Streatfield SJ: Plant-made vaccines and biopharmaceuticals. Trends in Plant Science, 14: 669-679 (2009)

11) Verma D, Samson N, Koya V, Daniell H: A protocol for expression of foreign genes in chloroplasts. Nature Protocols 3: 739-758 (2008).

12) Lee SM, Kang K, Chung H, Yoo SH, Xu XM, Lee SB, Cheong JJ, Daniell H, Kim M: Plastid Transformation in the Monocotyledonous Cereal Crop, Rice and Transmission of Transgenes to Their Progeny. Mol. & Cells 21: 401-410 (2006).

13) Cui C, Song F, Tan Y, Zhou X, Zhao W, Ma F, Liu Y, Hussain J, Wang Y, Yang G, He G: Stable chloroplast transformation of immature scutella and inflorescences in wheat. Arch Biochim Biophys Sin 43: 284-291 (2011)

14) Kausch AP, Hague Oliver JM, Li Y, Daniell H, Maschia P, Watrud LS, Stewart CN Jr: Transgenic biofuel feedstocks and strategies for bioconfinement. Biofuels 1:163-176 (2010).

15) Ruiz O, Daniell H: Engineering cytoplasmic male sterility via the chloroplast genome. Plant Physiology 138: 1232-1246 (2005)

Gene Flow Control in Forest Trees: Approaches and Progress

Steven H. Strauss[1]*, Chunsheng Zhang[2], and Maud Hinchee[2]

[1]* Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon 97331-5752. Steve.Strauss@OregonState.Edu

[2] ArborGen Inc., 180 Westvaco Road, Summerville, South Carolina 29483

Abstract

Because of their limited state of domestication, the capability of most species to disperse pollen and often also seeds widely, and regulatory and marketplace requirements, genetic sterility may be an enabling technology for many applications of transgenic forest biotechnology. We review the main options for genetic engineering of containment in forest trees, outline their state of development, and then present results from field studies of pollen ablation in poplar, eucalypts, and pine. Results to date show that ablation, though the first method for producing transgenic sterility, can be highly effective in diverse tree species in the field. With the addition of new promising methods, such as multiple gene suppression and directed genetic mutation, genetic containment technologies for forest trees are becoming a versatile biological reality.

Purpose-grown transgenic trees for the traditional forest industry as well as the emerging renewable energy industry are being developed and tested in order to improve the sustainability and cost-effectiveness of producing woody biomass (Hinchee et al. 2010; Nehra et al. 2005). Genetic sterility has been long discussed as the major option for mitigating the spread of transgenes in forest trees (e.g., Strauss et al . 1995). Concerns over the spread of transgenes derive from ecological considerations of genes that provide novel traits and the great difficulty in predicting their future ecological impacts (James et al. 1998), and legal and market issues from spread of transgenes into wild or GMO-free certified forests (Strauss et al. 2001). Because intensively grown forest trees are commonly vegetatively propagated—and seeds as well as pollen can often move far beyond plantations—there has been considerable interest in producing completely sterile trees. This may, at least in theory, be simpler and provide more complete sterility than generating plants that are sterile in fields but can be induced to revert for seed-based propagation.

However, the most advanced efforts to date have been around male-sterility, as pollen moves furthest and the technology for producing transgenic male-sterility is most advanced, having been first demonstrated in 1990 (Mariani et al. 1990). These methods used genetic ablation, where a reproductive tissue predominant promoter drives the expression of a cytotoxic gene such as an RNAse (e.g., barnase) or a ribosomal inhibitor, thus disrupting specific developmental lineages such as pollen formation. Thus, it is no surprise that the earliest demonstrations of the effectiveness of genetic sterility genes in tree species have been based on ablation methods (e.g., Skinner et al. 2003, Brunner et al. 2007; Figure 1). A modification of the ablation method reported by Mariani et al. in 1990 has been utilized successfully to achieve what appears to be complete pollen control in two major commercial forest tree genera, *Pinus and Eucalyptus* (Figure 2). As we will show, this level of control was observed in multiple transgenic lines of each genus, in multiple constructs in which the pollen control gene was stacked with other genes of interest, and in multiple multi-year field trials. The growth and development of the pollen ablated trees appeared similar to control trees (Zhang and Hinchee, unpublished results). The primary modification of the Mariani approach was to utilize a pine tapetal specific promoter driving a coding sequence for a mutant barnase enzyme with very low activity—thus apparently avoiding negative effects on growth and *in vitro* regenerability.

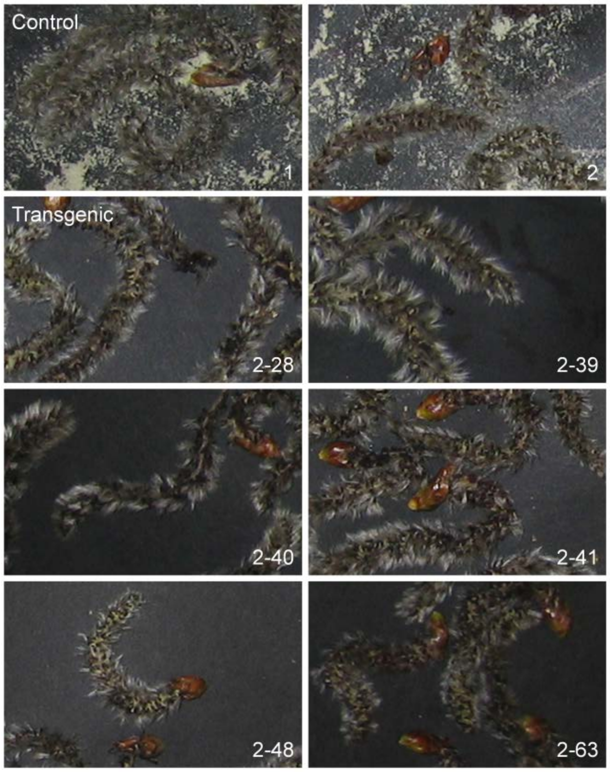


Figure 1. Dehiscing catkins from field-grown male transgenic poplars collected in a Petri dish. The transgenic trees had an ablation based male-sterility gene similar to that reported in Mariani et al. (1990); the non-transgenic control trees were of the same poplar genotype (top two images). Note the visible release of pollen only from the control catkins. Modified from Brunner et al. (2007).

In addition to ablation methods, , other viable methods that have strong support in model plant species as to their likely effectiveness include suppression of the transcription or protein function of genes such as transcription factors that are essential for reproductive development. This includes widely used approaches such as RNA interference—which are being intensively studied in poplar in the Strauss laboratory and will be briefly described—and less commonly used approaches such as production of proteins with dominant negative amino acid substitutions or suppressor amino acid motifs (reviewed in Brunner et al. 2007).

A new approach is to specifically mutate such genes essential for reproduction is by zinc finger or other directed mutagenesis approaches, which appear to have high efficiency at gene targeting and mutation (e.g., Shukla et al. 2009). A major project to accomplish this in poplar has just started in the Strauss laboratory and will be briefly described. Finally, an alternative approach to floral sterility is to induce excision of transgenes during gametogenesis, thus reproduction should otherwise be normal. High levels of efficiency for pollen-associated excision have been previously reported (Moon et al. 2009), though we are unaware of field demonstrations of its efficacy.

We will discuss the reproductive biology of forest trees, the rationale for developing sterility systems for native and exotic species, the regulatory and technical obstacles to research and commercial use, and show examples of our ongoing research and successes.

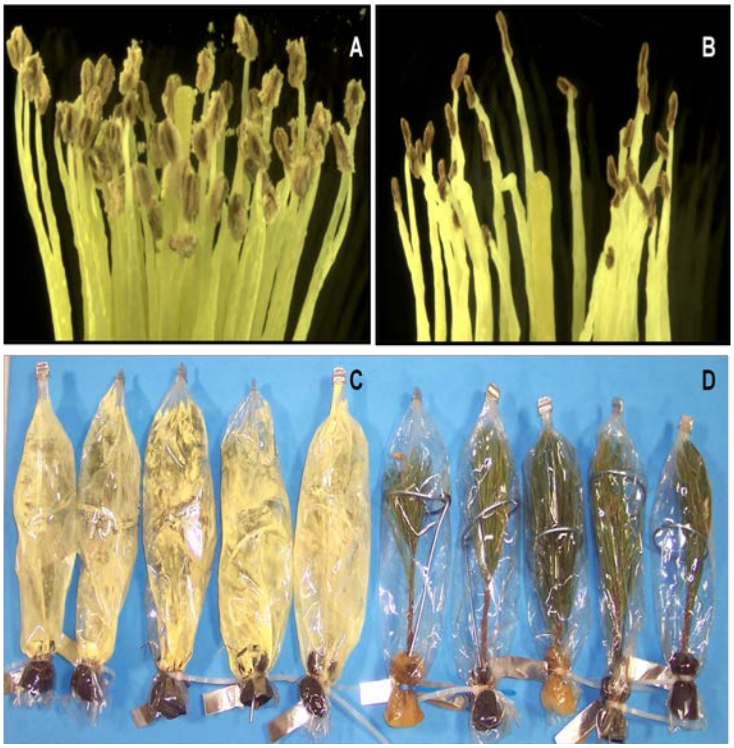


Figure 2. Dehiscing male reproductive structures of *Eucalyptus occidentalis* and *Pinus taeda*.
A: Anthers of a wild type greenhouse grown Eucalyptus. B: Anthers of transgenic greenhouse grown Eucalyptus transformed with pollen ablation gene consisting of a mutant barnase driven by a pine tapetal-specific promoter. C: Bagged branches, each bearing multiple male cones, obtained from non-transgenic pine grafted onto 7 year-old field grown pine. D: Bagged branches, each bearing multiple male cones, obtained from transgenic pine containing the same pollen ablation gene used in Eucalyptus and which were grafted onto field grown pine.

References

Brunner, A., J. Li, S. DiFazio, O. Shevchenko, R. Mohamed, B. Montgomery, A. Elias, K. Van Wormer, S.P. DiFazio and S.H. Strauss. (2007) Genetic containment of forest plantations. *Tree Genetics & Genomes 3*: 75-100.

Hinchee, M., L. Mullinax, and W.H. Rottmann. (2010) Woody Biomass and Purpose-Grown Trees as Feedstocks for Renewable Energy. *In:* Plant Biotechnology for Sustainable Production of Energy and Co-products; Biotechnology in Agriculture and Forestry. *66*: 155-208.

James, R.R., DiFazio, S.P., Brunner, A.M., and Strauss, S.H. (1998) Environmental effects of genetically engineered woody biomass crops. *Biomass & Bioenergy 14:* 403-414

Mariani C., Beuckeleer M.D, Truettner J., Leemans J., and Goldberg R.B. (1990) Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature *347*: 737-741.

Moon, H.S., Y. Li, and C.N. Stewart Jr. (2009) Keeping the genie in the bottle: transgene biocontainment by excision in pollen. Trends Biotechnology *28*: 3-8.

Nehra, N.S., M.R. Becwar, W. \H. Rottmann, L. Pearson, K. Chowdhury, S. Chang, H.D. Wilde, R.J. Kodrzycki, C. Zhang, K.G. Gause, D.W. Parks, & M.A. Hinchee, M.A. (2005). Forest Biotechnolgy: Innovative Methods, Emerging Opportunities. *In Vitro Cell. Dev. Biol.—Plant 41*: 701–717.

Shukla, V.K., Doyon, Y., Miller, J.C., Dekelver, R.C., Moehle, E.A., Worden, S.E., Mitchell, J.C., Arnold, N.L., Gopalan, S., Meng, X., Choi, V.M., Rock, J.M., Wu, Y.Y., Katibah, G.E., Zhifang, G., McCaskill, D., Simpson, M.A., Blakeslee, B., Greenwalt, S.A., Butler, H.J., Hinkley, S.J., Zhang, L., Rebar, E.J., Gregory, P.D., and Urnov, F.D. (2009). Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases. Nature *459***:** 437-441.

Skinner, J.S., Meilan, R., Ma, C., and Strauss, S.H. (2003) The Populus PTD promoter imparts floral-predominant expression and enables high levels of organ ablation in Populus, Nicotiana and Arabidopsis. *Molecular Breeding 12:* 119-13.

Strauss, S.H., Rottmann, W.H., Brunner, A.M., and Sheppard, L.A. (1995) Genetic engineering of reproductive sterility in forest trees. *Molecular Breeding 1:* 5-26.

Strauss, S.H., Coventry, P., Campbell, M.M., Pryor, S.N., and Burley, J. (2001) Certification of genetically modified forest plantations. *International Forestry Review 3(2):* 85-102.