Gene Flow Among Transgenic Plants and Their Wild Relatives: Implications for Risk Assessment

Proceedings of the Gene Flow Symposium of the North Central Weed Science Society Annual Meeting

(www.ncwss.org)

Dates:	December 12 & 13, 2007
Location:	The Hyatt Regency, St. Louis, Missouri, USA
Purpose:	The purpose of the meeting is to bring together academic, industry, government, and other interested scientists to discuss recent and ongoing research on topics related to gene flow from transgenic plants. The meeting will focus on: 1) within-species gene flow; 2) hybridization and gene introgression between transgenic plants and their sexually compatible relatives; 3) consequences of gene flow from transgenic and non-transgenic plants; 4) approaches to managing gene flow; and 5) modeling gene flow.

Organizing Committee:

Michael Horak, Chair. Monsanto Company (michael.j.horak@monsanto.com)
David Gealy, USDA, (dgealy@spa.ars.usda.gov)
Hector Quemada, Crop Technology, Inc.(hdquemada@croptechnology.com)
Allison Snow, Ohio State Univ. (snow.1@osu.edu)
Neal Stewart, Univ. of Tennessee (nealstewart@utk.edu)
Mark Westgate, Iowa State Univ., (westgate@iastate.edu)

Gene Flow Symposium – Wednesday

Room: *Grand Ballroom B*
Moderators: Allison Snow, Hector Quemada

10:00 **Welcome and Introduction.** Michael Horak, Monsanto Company, St. Louis, MO.

10:15 **Seed Mediated Gene Flow in Canola**. Linda M. Hall* and Robert Gulden, University of Alberta, Edmonton, AB. (128)

11:15 **Pollen Mediated Gene Flow in Canola.** Hugh Beckie* and Linda Hall, Agriculture and Agri-Food Canada, Saskatoon, SK and University of Alberta, Edmonton, AB. (129)

12:15 **Lunch Break**

1:45 **Interspecific Gene Flow in Canola.** Suzanne I. Warwick*, Agriculture and Agri-Food Canada (AAFC), Ottawa, ON, Canada. (130)

2:45 **Impact of Distinct Insect Pollinators on Gene Flow.** Johanne Brunet* and Karsten G. Holmquist, USDA-ARS, University of Wisconsin, Madison. (131)

3:00 **Ecological Effects of Virus-Resistant Transgenic Squash on Wild Squash Population Dynamics.** Holly R. Prendeville* and Diana Pilson, University of Nebraska, Lincoln, NE. (132)

3:15 **Long-term Field Studies of the Evolution of Crop-Weed Hybrids in Radish: Implications for Invasiveness.** Allison A. Snow*, Lesley G. Campbell, Theresa M. Culley, and Caroline E. Ridley, The Ohio State University, Columbus, OH; University of Maryland, College Park, MD; University of Cincinnati, OH; and University of California, Riverside, CA. (133)

3:30 **Discussion**

3:45 **Break**

4:00 **Weed-to-Weed Gene Flow – What is the Potential for Glyphosate Resistance Movement via Interspecific Hybridization?** Micheal D. K. Owen* and Ian A. Zelaya, Iowa State University, Ames, IA and Syngenta Ltd. Jealott's Hill International Research Centre, UK. (134)

4:15 **Gene Flow and Risk Assessment: Case by Case Considerations.** Michael J. Horak* and Thomas E. Nickson, Monsanto Company, St. Louis, MO. (135)

4:30 **Gene Flow Dynamics and Confinement: A Regulatory Perspective.** Subray Hegde*, Biotechnology Regulatory Services/APHIS/USDA, Riverdale, MD. (136)

4:45 **Introduction of a New Issue Paper from CAST, 'Implications of Gene Flow in the Scale-up and Commercial Use of Biotechnology-Derived Crops: Economic and Policy Considerations'.** David R. Gealy*, USDA-ARS, DBNRRC, Stuttgart, AR. (137)

5:15 **Discussion**

5:30 **Adjourn**

6:45 **Banquet**

Gene Flow Symposium - Thursday

Room: *Grand Ballroom B* Moderators: Mark Westgate, David Gealy

8:15 **Modeling the Biology of Out-Crossing by Adventitious Pollen.** Mark Westgate*, Juan Astini, Agustin Fonseca, Jon Lizaso, Craig Clark, and Ray Arritt, Iowa State University, Ames, IA. (199)

8:30 **Application of a 3D Windbreak Model to Compare Field Plot Designs for Limiting Pollen Dispersal.** Craig Clark, Juan Astini, Ray Arritt*, Mark Westgate and Susana Goggi, Iowa State University, Ames, IA. (200)

8:45 **Application of Large Eddy Simulation to Quantify Dispersal of Viable Maize Pollen.** Brian Viner*, Ray Arritt, Mark Westgate and Susana Goggi, Iowa State University, Ames, IA. (201)

9:00 **Pollen Flow in the Environment - Development of a Research Program.** John A. Glaser*, USEPA, Office of Research & Development, National Risk Management Research Laboratory, Cincinnati, OH. (202)

9:15 **Seed-to-Seed and Hay-to-Seed Pollen Mediated Gene Flow in Alfalfa.** Larry R. Teuber*, Shannon Mueller, Allen Van Deynze, Sharie Fitzpatrick, James R. Hagler and Jose Arias, University of California, Davis, Forage Genetics, Inc, West Salem, WI and ARS-USDA, Arid-Land Agricultural Research Center, Maricopa, AZ. (203)

9:30 **Alfalfa Gene Flow Research and Information: Applicability to Seed Production Systems.** Sharie Fitzpatrick*, Jose Arias, Mark McCaslin and Peter Reison, Forage Genetics, Inc., West Salem, WI. (204)

9:45 **Discussion**
10:00 **Break**

10:15 **Red Rice Diversity and Planting Date Effects on Risk of Gene Flow.** Nilda R. Burgos*, Vinod K. Shivrain, David R. Gealy, Kenneth L. Smith, and Robert C. Scott, University of Arkansas, Fayetteville, AK and USDA-ARS, DBNRRC, Stuttgart, AK. (205)

10:30 **Gene Flow between Sugar Beet and Weed Beet: From Facts to Models.** Henri Darmency*, Nathalie Colbach, Mathilde Sester, Yann Tricault, Etienne Klein and Marc Richard-Molard, INRA, Dijon, France. (206)

10:45 **Discussion**

11:00 **Commercial-Scale Pollen-Mediated Gene Flow in Winter Wheat in the Central Western Great Plains.** Todd A. Gaines, Patrick F. Byrne*, Philip Westra, Scott J. Nissen and Phillip L. Chapman, Colorado State University, Fort Collins, CO; W. Brien Henry, USDA-ARS, Akron, CO; and Dale L. Shaner, USDA-ARS, Fort Collins, CO. (207)

11:30 **Hybridization between Jointed Goatgrass and Imidazolinone-Resistant Winter Wheat.** Todd A. Gaines,* W. Brien Henry, Patrick F. Byrne, Philip Westra, Scott J. Nissen and Dale L. Shaner, Colorado State University, Fort Collins, CO; USDA-ARS, Akron, CO; and USDA-ARS, Fort Collins, CO. (208)

11:45 **Tier 1 Evaluation of Pollen-Mediated Gene Flow between Triticale (X Triticosecale, Wittmack) and Common Wheat, Durum Wheat and Rye.** Melissa J. Hills*, Linda M. Hall and François Eudes, Agriculture and AgriFood Canada, Lethbridge, Alberta; Alberta Agriculture and Food/University of Alberta, Edmonton; Grant MacEwan College, Edmonton, Alberta. (209)

12:00 Discussion and Wrap-up

Poster Titles –

Identifying Maize Diversity Areas and Implications Regarding Biosafety Measures. Francisca Acevedo*, José Sarukhán, Jorge Larson, Elleli Huerta, Patricia Koleff, Claudia Aguilar, Alejandra Barrios and Oswaldo Oliveros, National Commission for the Knowledge and Use of Biodiversity, CONABIO, Mexico City, Mexico and Ministry of the Environment and Natural Resources, Mexico City, Mexico (66)

Seed-Mediated Gene Flow in Certified and Farm-Saved Seed Lots. Todd A. Gaines*, Christopher Preston, Patrick F. Byrne, W. Brien Henry, and Philip Westra, Colorado State University, Fort Collins; University of Adelaide, Australia; and USDA-ARS, Akron, CO. (67)

Interloper's Legacy: Invasive, Hybrid-Derived California Wild Radish (Raphanus sativus) Evolves to Outperform its Immigrant Parents. Caroline E. Ridley*, Rosamond F. Tsao and Norman C. Ellstrand, University of California, Riverside, CA. (68)

Sympatry and Hybridization of Canola and Bird Rape (Brassica rapa) in Quebec. Marie-Josée Simard, Anne Légère, and Suzanne I. Warwick*, Agriculture and Agri-Food Canada (AAFC) Québec, QC; Saskatoon, SK; and Ottawa, ON, Canada. (69)

Do Escaped Transgenes Persist in Nature? The Case of an Herbicide Resistance Transgene in Weedy Population of Brassica rapa. Suzanne I. Warwick*, Anne Légère, Marie-Josée Simard, and Tracey James, Agriculture and Agri-Food Canada (AAFC) Ottawa, ON; Saskatoon, SK; Québec, QC; and Ottawa, ON, Canada. (70)

Measuring the Effects of Crop Genetic Load on Productivity and Fitness in Weedy Brassica rapa (wild turnip) × Brassica napus (oilseed rape) Hybrid Populations. Reginald J. Millwood*, Christy W. Rose*, and C. Neal Stewart, Jr., University of Tennessee, Knoxville, TN. (71)

Strategies to Reduce Transgene Movement and Persistence. Hong S. Moon*, Jason N. Burris*, Reginald J. Millwood, Christy W. Rose, and C. Neal Stewart, Jr., University of Tennessee, Knoxville, TN. (72)

Estimating Pollen-Mediated Gene Flow in Colorado Corn Fields with the Blue Kernel Trait. Patrick F. Byrne*, Todd A. Gaines, Ron F. Meyer, and Rob Alexander, Colorado State University, Fort Collins, CO; Colorado State University, Burlington, CO; Boulder County Parks and Open Space Department, Longmont, CO. (73)

Assessment of Potential Impact of Hybridization between Teosinte (Zea ssp.) and Maize (Zea mays ssp. mays) on Dormancy Characteristics of Teosinte. Baltazar M. Baltazar*, William J. Duncan, Daniel L. Kendrick and Michael J. Horak, Monsanto Company, St. Louis, MO. (74)

Regulation of Diurnal Pollen Release. Brian Viner*, Ray Arritt and Mark Westgate, Iowa State University, Ames, IA. (75)

Variation in ALS Herbicide Resistance of Diverse Crop-Wild Sunflower Hybrids. Kristin L. Mercer*, Kevin Betts, Ruth G. Shaw, and Donald L. Wyse, The Ohio State University, Columbus, OH and University of Minnesota, St. Paul, MN. (76)

Lifetime Fecundity of F1 Crop-Wild Sorghum Hybrids: Implications for Gene Flow from Transgenic Sorghum in Africa. Allison A. Snow, Patricia M. Sweeney*, Cécile Grenier, Tesfaye Tesso, Issoufrou Kapran, Gurling Bothma, Gebisa Ejeta, and Jeffrey F. Pedersen, The Ohio State University, Columbus, OH; Purdue University, West Lafayette, IN; Ethiopian Institute of Agricultural Research, Nazareth, Ethiopia; Institut National de la Recherche Agronomique du Niger, Niamey, République du Niger; ARC-Roodeplaat, Pretoria, South Africa; and USDA, ARS, University of Nebraska, Lincoln, NE. (77)

Crop-Wild Hybridization and the Rate of Evolution in Weeds. Lesley. G. Campbell*, Allison A. Snow, and Patricia M. Sweeney, University of Maryland, College Park, MD and The Ohio State University, Columbus, OH. (78)

Modeling Pollen Dispersal between Fields of White Clover – Co-existence with GM-White Clover? Christina L. Løjtnant*, Christian. F. Damgaard and Rikke B. Jørgensen, Risø National Laboratory; Technical University of Denmark, DK-4000 Roskilde, DMU; and University of Århus, DK-8600 Silkeborg, Denmark. (79) CANCELLED

Biosafety Assessment and Benefits for Co-existence of Biological Contained Plants – Regulatory Assessment in the EU-project "TransContainer". Christiane Koziolek* and Detlef Bartsch, Federal Office of Food Safety and Consumer Protection, Berlin, Germany. (80)

Addressing Gene Flow Issues in Cowpea for West Africa. Remy Pasquet, ICIPE, IRD Nairobi, Kenya; Barry Pittendrigh, Purdue University, West Lafayette, IN; Mohammad Ishiyaku, Ahmadu Bello University, Zaria, Nigeria; Ibrahim Baoua, INRAN, Maradi, Niger; Clementine Dabiré, INERA Ouagadougou, Burkina Faso; Malik Ba, INERA, Ouagadougou, Burkina Faso; Manuele Tamò, IITA Cotonou, Benin; Larry Murdock, Purdue University, West Lafayette, IN; and Joseph Huesing*, Monsanto Company, St. Louis, MO. (81)

ABSTRACTS OF PRESENTATIONS AND POSTERS

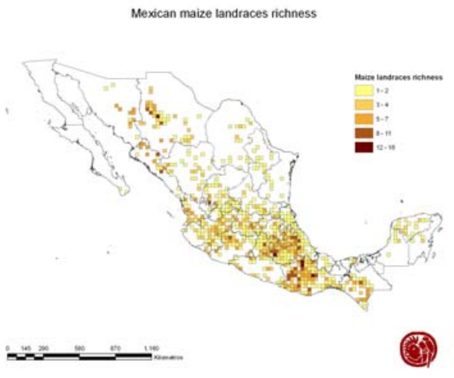
Listed alphabetically by first author; also listed at www.ncwssa.org by scheduling number.

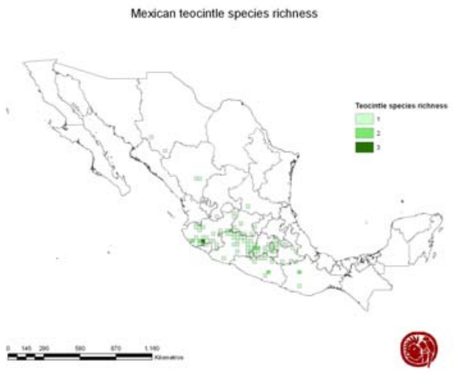
IDENTIFYING MAIZE DIVERSITY AREAS AND IMPLICATIONS REGARDING BIOSAFETY MEASURES. Francisca **Acevedo**, José Sarukhán, Jorge Larson, Elleli Huerta, Patricia Koleff, Claudia Aguilar, Alejandra Barrios and Oswaldo Oliveros*, National Commission for the Knowledge and Use of Biodiversity, CONABIO, Mexico City, Mexico and Ministry of the Environment and Natural Resources, Mexico City, Mexico.

In March 2005, Mexico approved the "Biosafety Law on Living Modified Organisms"(visit http://bch.cbd.int/database/record.shtml?id=8474) which among other things calls for a special regime to protect maize (article 2 fraction XI) and specifies the need to identify areas that are center of origin and current centers of genetic diversity for those species for which Mexico is center of origin and/or center of genetic diversity (articles 86 and 87), so as not to release GM related species in these areas.

Article 86 calls for the Ministries of Agriculture and Environment to arrange for "legal agreements" regarding these areas based on existing information coming from several governmental institutions, among those, the National Commission for the Knowledge and Use of Biodiversity (CONABIO, visit at www.conabio.gob.mx). In 2006 the Ministry of Agriculture asked CONABIO to provide the existing information on maize and its related wild relatives present in its biological databases. CONABIO generated a document called "General elements to determine the maize origin and genetic diversity centers, and the specific case of the experimental release into the environmental of transgenic maize in Mexico" (visit http://www.conabio.gob.mx/conocimiento/bioseguridad/doctos/Doc_CdeOCdeDG.pdf) which was sent to the two Ministers as information for the decision making process related to this particular issue.

This document includes distribution analysis both for maize landraces and teocintle species in a quadrant system of 0.5° x 0.5° (approximately 25 km per side) were landrace and species numbers present are enumerated. See maps that include all maize and teocintle information available to CONABIO through its biological databases.





It also includes four possible decision making scenarios related to spatial distribution, going from less to more conservative, the most conservative being a 32 km buffer around the quadrant according to *Luna et al.* 2001 as a safety area related to the maximum hypothetical linear distance of pollen flow. Article 86 calls for current genetic diversity areas, what this means is not clear. CONABIO decided that information coming from 1990 and onward would be considered current under its analysis, whilst only 20% of the data deposited in its biological databases fitted the criteria.

The document makes recommendations, one of them being to "integrate all the existing information in the country as well as to update it to reduce uncertainty in delimitating the areas the law calls for". Taking this recommendation into account, the two ministries plus CIBIOGEM, a interministerial commission on biosafety (visit http://www.cibiogem.gob.mx) donated funds so as to accomplish the task asked for in this recommendation.

The key question is if delimitating these areas will be enough so as to make sure that maize landraces and/or teocintle species present in these areas will be "protected" from GMO related species if grown in Mexican territory. Probably not. Even though buffers were chosen to be the most "conservative" possible, no biosafety measures will be enough at a commercial scale as to counteract traditional seed exchange between the rural farmers which happens to be the single most important factor to ensure genetic diversity existing in the first place. The law in question on the other hand does not describe the components of the regime. Delimitating areas will certainly be a component but seed management and other cultural practices should be considered. This is why an exercise to map land use to reflect the distribution of agroindustrial production (indicated by the presence of irrigation agriculture) and traditional rainfed agriculture would be useful as well in such an analysis.

Although the analysis here presented focuses on maize, the fact is that GMO have been developed and trials are underway for other crops for which Mexico is a center of origin and/or center of genetic diversity. If other genera are added to the analysis what would emerge are centers of diversity of genetic resources for food and agriculture. These areas, viewed as regions which contribute to *in situ* conservation of genetic resources could then be subject to specific policies that promote and protect this diversity.

Luna S., Figueroa J., Baltazar B., Gómez R., Townsend R., J.B. Schoper. Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Science 2001 Vol. 41, pp 1551-1557.
Turrent A. and J.A. Serratos. Context and Background on Maize and its Wild Relatives in Mexico. Chapter 1. In: Maize and Biodiversity: The Effects of Transgenic Maize in Mexico. CEC, 2004.
Biosafety Law on Living Modified Organisms (visit http://bch.cbd.int/database/record.shtml?id=8474)
"General elements to determine the maize origin and genetic diversity centers, and the specific case of the experimental release into the environmental of transgenic maize in Mexico" (visit http://www.conabio.gob.mx/conocimiento/bioseguridad/doctos/Doc_CdeOCdeDG.pdf)

ASSESSMENT OF POTENTIAL IMPACT OF HYBRIDIZATION BETWEEN TEOSINTE (*Zea* spp.) AND MAIZE (*Zea mays* spp. *mays*) ON DORMANCY CHARACTERISTICS OF TEOSINTE. Baltazar M. **Baltazar***, William J. Duncan, Daniel L. Kendrick and Michael J. Horak, Monsanto Company, St. Louis, MO 63167, USA.

Teosinte (*Zea* spp.) is an annual and perennial grass endemic to Mexico and Central America. Teosinte resembles maize (*Zea mays* spp. *mays*), but differs in various phenotypic characteristics including: pollen size and pollen viability, number of tassels per plant, and in the morphology of the pistillate inflorescence. In contrast to maize, teosinte populations survive as wild plants. This may be in part due to seed dispersal and dormancy mechanisms found in teosinte but absent in maize.

Research has demonstrated that gene flow and hybridization between teosinte and maize is possible. Furthermore, with the advent of genetically modified (GM) maize, questions have been raised regarding the potential ecological risks associated with the introduction of GM maize into areas where teosinte is present. However, there has been limited research on the biological effects of maize genes transferred to teosinte. One area of interest is on the effect of hybridization and introgression on seed dormancy (e.g., hard seed). One possible effect of hybridization between teosinte and maize would be decreased dormancy of hybrid seed.

Experiments to evaluate seed dormancy of *Zea* spp. were conducted during 2007. Four replicates of 25 seeds each of 8 *Zea* spp. were placed in rolled germination towels, arranged in a completely randomized block design and then placed in a germination chamber set at 25°C for 8 days. Seed/seedlings were evaluated as germinated (normal and abnormal, dead, or hard following AOSA guidelines 5 and 8 days after planting. The percentages of each category were statistically compared for each species.

Results of the experiments revealed four significantly different groups for the *Zea* species evaluated according to their percentage of dormant seed; Group 1, *Zea nicaraguensis* with 55%; Group 2, *Zea luxurians* (34%)*, Zea mays* spp. *mexicana* (30%) *and Zea mays* spp. *parviglumis* (25%); Group 3, *Zea huehuetenangensis* (20%) *and Zea perennis* (16%) and Group 4, *Zea diploperenis* (8%) *and Zea mays* spp. *mays* (0%).

Additionally, experiments are underway to evaluate seed dormancy characteristics of hybrid seed from crosses between teosinte and maize.

POLLEN-MEDIATED GENE FLOW IN CANOLA. Hugh J. **Beckie** and Linda M. Hall, Plant Scientist, Agriculture and Agri-Food Canada, Saskatoon, SK S7N 0X2 and Research Scientist/Adjunct Professor, Alberta Agriculture and Food, University of Alberta, Edmonton, AB T6G 2P5.

Outcrossing in canola or oilseed rape (*Brassica napus* L.) is highly variable, averaging 30%. The crop is partially pollinated by insects, particularly honey bees and bumble bees, but is also known to release large amounts of air-borne pollen. There is consensus that insects can be important contributors to short-distance pollination; in addition, bees and other insects such as pollen beetles can also contribute to long-distance pollen movement. However, the relative importance of wind vs. insects in long-distance gene flow in canola is uncertain. Coexistence among transgenic and non-transgenic cropping systems and identity preservation at the field level are increasingly important issues in many countries. Different types of pollen-mediated transgene flow models for canola have been released during the past decade primarily as a decision-support tool to achieve the European Union (EU) 0.9% transgenic labeling threshold for adventitious presence (AP) of authorized transgenes in food and feed. Many empirical models simulate gene flow well, although their utility is usually restricted by datasets with limited environmental variability or spatial scale. Development of predictive mechanistic models and simulation of transgene flow via insects and wind across agroecosystem landscapes are still in their infancy, although recent progress is promising. Experimental and modeling outcrossing studies reveal that no isolation distance is required between transgenic pollen donor and non-transgenic (conventional) receptor fields of realistic size to meet the EU threshold if AP from other sources (e.g., volunteers, admixture) is minimal. Because seed loss and volunteerism are common in canola, however, transgene flow via seed, not pollen, may be a greater source of AP.

IMPACT OF DISTINCT INSECT POLLINATORS ON GENE FLOW. Johanne **Brunet** and Karsten G. Holmquist, research ecologist and postdoctoral associate, United States Department of Agriculture, Agricultural Research System, Vegetable Crops Research Unit, University of Wisconsin, Madison, WI 53706.

The vast majority of fruits and vegetables, together with some hay crops (alfalfa) and some oil-producing crops (canola) are pollinated by insects. However we have little information on how insect pollinators affect the movement of genes via pollen and even less on how distinct insect pollinators may differentially affect pollen flow. In this study we examined whether two types of insect pollinators, bumble bees and hawkmoths, differentially affected gene flow via pollen in the rocky mountain columbine, our model system. In one experiment, we used paternity analyses to contrast the movement of genes via pollen by bumble bees and hawkmoths within and between patches within a population. In a second experiment, we genotyped seeds from many target females located within a 40 km^2 area, and used the Kindist module of Poldisp v.1.0 to fit the exponential power model to the haplotype data in order to calculate the average distance, axial variance and kurtosis of pollen dispersal for each pollination treatment. Both pollinator types were as efficient at moving pollen around (male function). In addition both pollinator types visited the same number of females and each female received similar progeny diversity whether pollen was carried by hawkmoths or by bumble bees. Moreover bumble bees did not limit their movement to nearest neighbor plants but frequently moved pollen among patches. On a larger geographical scale, dusk and night flying pollinators (hawkmoths) moved pollen 2-5 X as far as day flying pollinators (bumble bees). Pollen dispersal was fat tailed with relatively high kurtosis indicating the importance of long distance gene dispersal.

RED RICE DIVERSITY AND PLANTING DATE EFFECTS ON RISK OF GENE FLOW. Nilda R. **Burgos**, Vinod K. Shivrain, David R. Gealy, Kenneth L. Smith, and Robert C. Scott, Associate Professor, Graduate Assistant, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72704, Plant Physiologist, Dale Bumpers National Rice Research Center, Stuttgart, AR 72160, Professor, University of Arkansas Cooperative Extension Service, Monticello, AR 71656, and Associate Professor, University of Arkansas Cooperative Extension Service, Lonoke, AR 72086.

Red rice (*Oryza sativa* L.) is a problematic weed in rice production worldwide. Red rice control is difficult with conventional herbicides due to its similar biology and physiology as cultivated rice. Herbicide-resistant (HR) rice provides a valuable tool for red rice management, but with a risk of transferring HR gene to red rice populations. Diversity in red rice populations mainly in flowering time, plant height, and sexual compatibility with cultivated rice and the wide window of planting time can affect the rate of HR gene transfer from rice to red rice. Thus, experiments were conducted to understand the effect of: a) red rice biotype, b) rice cultivar, and c) sexual compatibility of rice and red rice on outcrossing rate.

Small plot experiments were conducted at the Rice Research Extension Center, Stuttgart; and Southeast Research and Extension Center, Rowher, AR from 2005 to 2007. Experimental design was a split-split plot with 3-4 replications, with planting date as main plot, Clearfield (CL) rice cultivar as subplot, and red rice biotype as sub-subplot. Rice and red rice were planted from early April to late May at 2-week intervals. CL161, CL hybrid and 12 red rice accessions were used. Red rice was planted in the middle row of each plot, flanked by four CL161 or CL hybrid rice on each side. Emergence, flowering, and plant height of red rice and CL rice were recorded. Red rice seed was harvested and a sub-sample of 100 g was planted in the field in subsequent years. Red rice seedlings were sprayed twice with imazethapyr at 0.14 kg ai/ha. Red rice plants which survived imazethapyr applications were counted and confirmed as outcrosses by DNA analysis. Manual crosses also were performed between the 12 red rice accessions and CL161 to determine their sexual compatibility.

The red rice accessions were 100 to 160 cm tall, with a flowering period ranging from 88 to 128, 87 to 117, 79 to 118, and 71 to 116 days after planting in the first, second, third, and fourth planting, respectively. Outcrossing rate differed between locations, but trends of outcrossing rate affected by red rice biotypes, CL rice, and planting dates were similar at both locations. At any given planting date, outcrossing rates differed between red rice accessions due to differences in flowering time. Planting date by CL cultivar and planting date by red rice accession interactions were significant ($p< 0.05$) for outcrossing rate. The outcrossing rate in different red rice accessions ranged from 0 to 0.3% across planting dates. Brownhull red rice had the highest outcrossing rate regardless of the CL rice cultivar pollen donor, and strawhull had the lowest outcrossing rate in general. Averaged over planting dates, the outcrossing rate between CL hybrid rice and red rice accessions was 0.3% compared with 0.06% in CL161. In experiments related to compatibility, brownhull, blackhull, and strawhull had 91, 78, and 71% seed set, respectively, which corroborate the results of field experiments. The data suggest that the interaction of planting date, red rice biotype, and rice cultivar can result in no gene transfer in some cases to significantly high risk of gene transfer in others. Hence, these factors need to be considered in planning HR gene transfer mitigation strategies for rice.

ESTIMATING POLLEN-MEDIATED GENE FLOW IN COLORADO CORN FIELDS WITH THE BLUE KERNEL TRAIT. Patrick F. **Byrne**, Todd A. Gaines, Ron F. Meyer, and Robert Alexander. Associate Professor, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523-1170, Graduate student, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, Extension Agronomist, Colorado State University Cooperate Extension, Burlington, CO 80807-1674, and Agricultural Resource Specialist, Boulder County Parks and Open Space, Longmont, CO 80503.

Pollen-mediated gene flow (PMGF) from genetically engineered (GE) corn (*Zea mays* L.) has become a topic of intense interest. Organic growers and others seeking to avoid the presence of transgenic material in their corn harvests want to know how far to isolate their crops from GE hybrids. Although similar studies on corn gene flow have been conducted in other parts of the U.S., to our knowledge none had been carried out under conditions similar to Colorado's corn growing areas. Our objective was to determine the percent cross-pollination that occurred across a range of distances in multiple locations and years in Colorado’s Front Range and eastern plains. We used the dominant blue kernel trait to track cross-pollination. Plots of blue corn were planted as central islands surrounded by large fields of yellow corn. Dates of pollen shed and silk emergence were recorded to verify a sufficient overlap in flowering time between pollen source and recipient plants. At harvest, samples of 10 ears each were collected in several directions from the blue corn plot at distances that generally ranged from 0.75 to 300 m. For each sample, the number of blue and yellow kernels were counted and the percentage of blue kernels was calculated as an indication of the frequency of cross-pollination. Data from a total of 13 locations over six years were used in the analysis. As expected, the amount of cross-pollination was high at the closest sampling sites (mean of 29.3% at 0.75 m). Cross-pollination decreased rapidly with distance, dropping to a mean of 0.20% at 46 m and 0.05% at 92 m. The farthest distance at which any blue kernels were observed was 320 m. The information collected in this study helped a Boulder County technical advisory committee determine an isolation distance of 46 m between GE corn crops and organic corn crops on county-owned Open Space lands. This study will also be relevant elsewhere in Colorado and similar environments where there are concerns about cross-pollination from GE corn.

CROP-WILD HYBRIDIZATION AND THE RATE OF EVOLUTION IN WEEDS. Lesley. G. **Campbell**, Allison A. Snow, and Patricia M. Sweeney, Postdoctoral fellow, Department of Plant Sciences and Landscape Architecture, University of Maryland, College Park, MD, 20742, Professor, Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, OH, 43210, and Senior Research Associate, Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, OH, 43210.

When species hybridize their offspring routinely suffer from reduced fertility and poorly adapted phenotypes. Consequently, it seems unlikely that these plants could be successful weeds. Reflecting this belief, risk assessments of crop-wild hybrids often dismiss the potential for crop gene flow to produce ‘superweeds’. However, in the absence of empirical evidence, the evolutionary potential of early-generation hybrids remains hypothetical. Here, we explore the potential for *rapid* evolution in crop-weed hybrids and its consequences for crop allele introgression. Using hybrids of a cosmopolitan weed (*Raphanus raphanistrum*) and its cultivated relative (*R. sativus*), we compared the ability of hybrid and wild lineages to respond to artificial selection for early flowering and large size at reproduction, two life-history strategies which characterize weedy species. *Raphanus raphanistrum* grows a rosette with a thin, fibrous taproot, bolts within a few weeks after germination and produces yellow flowers soon after. On the other hand, crop breeding has emphasized delayed bolting with white flowers in *R. sativus* in order to produce the edible, enlarged roots (Snow and Campbell, 2005). Early flowering may be adaptive for weedy radishes because growing seasons for weeds are often curtailed by tilling schedules, herbivores, frost, and other causes of mortality or severe stress. The evolutionary potential to evolve earlier flowering may be more important for hybrid radishes given that hybrid fecundity, relative to wilds, may be limited by delayed flowering, a trait inherited from their cultivated parent and by low pollen fertility due to a reciprocal translocation that affects chromosome pairing (Snow et al. 2001; Campbell and Snow, in prep.). When hybridization occurs between species with such diverse life histories, the individual offspring will be phenotypically variable. Populations created with this initial diversity should have the opportunity to evolve along diverse trajectories with respect to life history. If crop-wild hybrids can evolve quickly from maladaptive intermediates to adaptive phenotypes, they may be more difficult to control. Large size in annual weeds is often correlated with rapid growth rates. In weedy radish, leaf length is correlated with high flower and seed production, suggesting that plants with rapid growth rates would also be highly fecund. If large size is adaptive, this may facilitate the introgression of additional adaptive quantitative traits into weed populations.

In wild and hybrid lineages, four generations of selection were performed to determine whether these traits exhibited a response to selection (i.e., were heritable) and the relative magnitude of their response across wild and hybrid lineages. Hybrid lineages exhibited a greater response to selection for early flowering suggesting its heritability is greater in hybrid lineages versus wild lineages. Early-generation hybrids had longer leaves than wild plants and they maintained this length difference after selection for longer leaves. This suggests that polygenic traits, such as size, inherited from domestic relatives may easily introgress into weed populations. Four generations of selection also resulted in the correlated evolution of hybrid flower petal color and hybrid pollen fertility. Large hybrid lineages exhibited higher than expected frequencies of plants with white petals, a crop-specific, simply inherited trait. Therefore, selection for a polygenic crop-specific trait accelerated the introgression of an additional crop-specific trait. Further, pollen fertility of early-flowering hybrid lineages was similar to that of wild lineages, and at least 12% higher than hybrid control lineages. Therefore, selection for earlier flowering in hybrid lineages led to rapid evolution of fertility, a key component limiting hybrid fitness. Despite selection for the early-flowering, wild phenotype, hybrid lineages maintained high frequencies of the crop-derived trait, white flower color, confirming persistent introgression. The persistence of white flower color and increase in pollen fertility after experimental manipulation of the selection environment may explain some results from our long-term studies in crop allele introgression (Snow et al., in prep; Campbell et al., 2006)

Both wild and hybrid lineages apparently possess substantial additive genetic variation for size at reproduction. Nevertheless, hybrid lineages evolved more rapidly under selection for age at reproduction and exhibited more extreme phenotypes under selection for large size at reproduction than their weedy parents. We suggest that hybrids have the potential to rapidly respond to newly invaded environments and may become more invasive weeds than their wild progenitors.

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APPLICATION OF A 3D WINDBREAK MODEL TO COMPARE FIELD PLOT DESIGNS FOR LIMITING POLLEN DISPERSAL.

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Placing windbreaks or shelter around a field may help reduce the escape of transgenes into the environment. An optimal configuration of such designs is desirable, but the cost and labor involved in field studies imposes practical limitations on the number of candidate designs that can be tested. We propose that a combined shelter flow model and a Lagrangian dispersion model can be used as a screening tool to test the effect of border design, field geometry, wind climatology, and other factors on pollen transport. This allows field studies to focus more efficiently on designs that are likely to be successful. We tested a the model by simulating results from field projects in the 2005 and 2006 growing seasons in which a tall annual grass (sorghum sudangrass) was planted as a border around a small maize plot. Field measurements for both 2005 and 2006 showed that a sorghum sudangrass border reduced the maximum distance of pollen dispersal from 300 m to 160 m. Model results show a decrease in downwind transport of pollen but predicted patterns of pollen deposition are much smoother than observed. We propose that this limitation derives partly from incomplete knowledge of the diurnal timing of pollen shed and partly from limitations in sampling observed pollen deposition.

GENE FLOW BETWEEN SUGAR BEET AND WEED BEET: FROM FACTS TO MODELS.

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Farm-scale monitoring was conducted at two locations in root production fields during six-years to study the occurrence and the mechanisms of gene flow between transgenic herbicide-resistant sugar beet and weed beet. We investigated the impacts of cultivar properties and control of bolting plants on the frequency of the transgene presence in weed beet populations. Specific experiments were carried out to quantify biological parameters affecting competition response, flowering, pollen flow, seed set and survival in the soil. These quantifications were incorporated as sub-models in the GENESYS model to simulate the effects of various farming systems (crop succession, cultivation techniques) on the dynamics and genetic composition of weed beet populations in a small region, and to propose the best agricultural practices to control weed beet and to prevent the advent of herbicide resistance in weed beet.

ALFALFA GENE FLOW RESEARCH AND INFORMATION: APPLICABILITY TO SEED PRODUCTION SYSTEMS.

S. **Fitzpatrick***, J. Arias, M. McCaslin and P. Reisen, Forage Genetics International, West Salem, WI 54669

With the advent of the first biotech-improved varieties in alfalfa, there have been renewed efforts to manage and model pollinator-mediated gene flow between alfalfa seed production fields. Separate research-scale models have been developed for honey bee (Teuber et al. 2005) and alfalfa leafcutting bee pollination systems (Fitzpatrick et al., 2003) which used plots ≤1 acre in most cases. Due to factors of scale and bee foraging behavior, etc, it is recognized that small alfalfa seed fields have greater proportional gene influx than larger fields. For example, certified foundation seed fields larger than 5 ac are required to have 600 ft isolation, whereas, smaller fields must have 900 ft (1.5X). Therefore, it was hypothesized that these research models were likely to overestimate the amount of pollen flow into commercial-scale fields. Nonetheless, FGI in consultation with alfalfa industry stakeholders used these small-field models and existing foundation standards to develop the FGI Best Practices for Roundup Ready Trait Stewardship during Commercial Seed Production ("Best Practices"). The Best Practices require isolation distances that are intentionally rigorous, science-based, and pollinator species-specific; the isolation distances are five to 95 times greater than certified standards. Specifically minimum distances are: 900 ft, 1 mi or 3 mi for leafcutter, alkali or honeybees, respectively. The practices were designed to help conventional seed growers mitigate adventitious presence (AP) to a very low amount (e.g., <0.5%). For a range of isolation distances, FGI used the RRA trait as a pollen-marker to compare pollen-flow predicted vs. observed values for 116 large fields grown 2006/2007. The new data were also used to determine the efficacy of the Best Practices seed stewardship program.

The amount of pollen-flow between large fields, in all cases, was less than was predicted using the models and well within the company's tolerance, <0.5%. In the 104 conventional seed lots grown with Best Practices minimum isolation, AP occurred infrequently and when detected, was at a very low level (0.004-0.180%). For the 12 closer fields, AP was ≤0.47%; the nearest field being only 120 ft isolated under leafcutter bee pollination. This nearest field (150 acres, 0.47% overall) was sampled on a grid and the borders were harvested separately to evaluate field border effects. As expected based on previous research and certification standards, the nearest border (the area <165 ft isolation) had the highest incidence of pollen-flow (>1%). For AP sensitive seed lots, segregated harvest of the nearest field edge can therefore be used to help mitigate gene flow to the main field bulked seed lot. Nearest-edge harvest segregation is consistent with seed certification standards for fields with a portion of the field at less than the minimum requirement, 165 ft isolation. Other FGI conventional seed lots were evaluated for seed admixture consequent to seed conditioning on equipment previously used for RRA seed handling; the equipment was thoroughly cleaned between lots. No AP was detected in these conventional seed lots.

While the research models are accurate for seed production in small fields (<5 ac) they significantly overestimate pollen-flow in the commercial setting where very large fields are common. The models have been extremely useful as a first step in the initial development of pollinator-specific commercial trait mitigation systems for alfalfa seed producers. These commercial-scale data help put in perspective the minimum incremental risk associated with potential "real world" concerns about pollination from wild pollinators, extraordinary pollen flow via wind-driven pollinator movement, and, contamination through physical mixtures of seed in harvesting and/or seed processing. It supports science-based isolation standards, the efficacy of the FGI Best Practices quality control program, and demonstrates that reasonable tools are available to allow successful coexistence within and between diverse alfalfa seed market sectors.

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Teuber, L.R., et al. 2005. Proc. of the NCWSS 2005 Meeting (http://www.ncwss.org/)

HYBRIDIZATION BETWEEN JOINTED GOATGRASS AND IMIDAZOLINONE-RESISTANT WINTER WHEAT.

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Gene flow between jointed goatgrass and winter wheat is a concern because transfer of herbicide resistance genes from imidazolinone-resistant (IR) winter wheat cultivars to jointed goatgrass could restrict weed management options for this serious weed of winter wheat cropping systems. The objective of this study was to investigate the frequency of interspecific hybridization between IR wheat and jointed goatgrass in eastern Colorado. Jointed goatgrass was sampled side-by-side with IR wheat and at distances up to 53 m away in both experimental plots and at commercial field study sites in 2003, 2004, and 2005. A total of 141 samples were collected from 16 different sites. A greenhouse screening method was used to identify IR hybrids in collected jointed goatgrass seed, and over 60,000 plants were screened. The average percent hybridization across sites and years when IR wheat and jointed goatgrass were grown side-by-side was 0.1% and the maximum was 1.6%. The greatest distance over which hybridization was documented was 16 m. The hybridization rate between wheat and jointed goatgrass will influence trait introgression into jointed goatgrass. Studies have subsequently been initiated to measure the frequency of hybrid plant backcrossing to jointed goatgrass under eastern Colorado field conditions.

SEED-MEDIATED GENE FLOW IN CERTIFIED AND FARM-SAVED SEED LOTS. Todd A. **Gaines**, Christopher Preston, Patrick F. Byrne, W. Brien Henry, and Philip Westra, Graduate student, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, Lecturer, School of Agriculture, Food, and Wine, University of Adelaide, South Australia, 5005, Associate Professor, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523-1170, Research Geneticist, USDA/ARS Corn Host Plant Resistance Research, Mississippi State, MS 39762, and Professor, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177.

Varietal purity in wheat seed production is necessary for agronomic uniformity and to enable potential market segregation. We conducted a survey of certified and farm-saved seed samples using a non-transgenic imidazolinone resistant (IR) winter wheat variety in 2004 and 2005 in eastern Colorado. The objective was to compare varietal purity based on type of seed producer and IR wheat history. Ninety-two samples of non-IR varieties were taken from certified and farm-saved seed growers, who either produced or had never produced IR wheat. Adventitious IR seeds were detected using a seed soaking technique in samples from each producer type and each IR production history. The total emerged plants evaluated for each sample ranged from 4,000 to 7,000. Levels of IR seed detected ranged from 0% to 11.28%. One certified sample and three farm-saved samples exceeded the 0.1% threshold for off-types in certified wheat seed. Using a two-factor analysis, farm-saved production class and positive IR history increased the estimated proportion of adventitious seed. Based on grower interviews, higher levels of adventitious seed presence were associated with volunteer plants from previous crops of the resistant variety and mechanical mixture during harvesting. Production practices for certified seed address these factors and may need to be strengthened if more stringent purity criteria are adopted. This information is important for risk assessment and policy development for potential commercial release of transgenic wheat varieties.

COMMERCIAL-SCALE POLLEN–MEDIATED GENE FLOW IN WINTER WHEAT IN THE CENTRAL WESTERN GREAT PLAINS. Todd A. **Gaines**, Patrick F. Byrne, Philip Westra, Scott J. Nissen, W. Brien Henry, Dale L. Shaner, and Phillip L. Chapman, Graduate student, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, Associate Professor, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523-1170, Professor, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, Professor, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, Research Geneticist, USDA/ARS Corn Host Plant Resistance Research, Mississippi State, MS 39762, Plant Physiologist, USDA/ARS Water Management Research, Fort Collins, CO 80526, and Professor, Department of Statistics, Colorado State University, Fort Collins, CO 80523-1877.

Pollen-mediated gene flow (PMGF) in wheat (*Triticum aestivum* L.) has been investigated in several studies, but most have been conducted on relatively small experimental plots. The introduction and widespread planting of a Clearfield (imidazolinone herbicide resistant) wheat cultivar in Colorado provided the opportunity to examine PMGF in commercial-scale production fields. We sampled a total of 56 large wheat fields in eastern Colorado in 2003, 2004, and 2005, and tracked the movement of the resistance gene from resistant cultivars to adjacent susceptible cultivars at distances up to 61 m. The highest level of PMGF we observed was 5.3% at 0.23 m, and the farthest sample at which we detected PMGF was 61 m. All 18 sampled cultivars showed some level of PMGF, with earlier heading cultivars having higher levels of cross-pollination than those that were late heading. At least in some cases, higher PMGF in early cultivars appeared to be due to late frosts that rendered recipient plants male sterile and therefore more susceptible to fertilization by foreign pollen. We used these data to develop a generalized linear mixed model with a random location effect. Based on the model results for cultivars heading earlier than the pollen source, the required separation distance between fields to ensure 95% confidence that 95% of locations have PMGF less than 0.9% is 41.1 m. For cultivars heading later than the pollen source, the equivalent required distance is 0.7 m. These are conservative confidence limits that should represent the highest levels of PMGF occurring in winter wheat in the central western Great Plains.

INTRODUCTION OF A NEW ISSUE PAPER FROM CAST--IMPLICATIONS OF GENE FLOW IN THE SCALE-UP AND COMMERCIAL USE OF BIOTECHNOLOGY-DERIVED CROPS: ECONOMIC AND POLICY CONSIDERATIONS. David R. **Gealy**, Kent J. Bradford, Linda Hall, Richard Hellmich, Alan Raybould, Jeffrey Wolt, and David Zilberman, United States Department of Agriculture–Agricultural Research Service, Dale Bumpers National Rice Research Center, Stuttgart, AR, Seed Biotechnology Center, Department of Plant Sciences, University of California, Davis, CA, Alberta Agriculture, Food and Rural Development/University of Alberta, Edmonton, Canada, United States Department of Agriculture–Agricultural Research Service, Corn Insects and Crop Genetics Research Unit, and Department of Entomology, Iowa State University, Ames, IA, Product Safety, Syngenta, Berkshire, United Kingdom, Biosafety Institute for Genetically Modified Agricultural Products, Iowa State University, Ames, IA, and Department of Agricultural and Resource Economics, University of California, Berkeley, CA.

This paper reviews the concept of gene flow—the successful transfer of genetic information between different individuals, populations, and generations (to progeny) and across spatial dimensions. The paper also discusses the relatively limited situations in which gene flow is likely to cause economic problems in the production of commercial biotech crops. Gene flow is presented in the context of an associated phenomenon, adventitious presence, in which unwanted substances unavoidably make their way into the production, channeling, and marketing system of grain and crop products.

Because reproductive biology differs markedly among crop species, so does the potential for outcrossing and subsequent gene flow. Economically or environmentally significant gene flow into weedy relatives of these crops often is limited because of restricted geographical overlap of the crop and weed regions or because the weedy relatives are not exceptionally competitive or invasive.

Numerous useful traits are being imparted into biotech (transgenic) and nonbiotech crops. Most of these traits are likely to have little impact on the dynamics of gene flow, especially outside of agricultural fields. Precommercialization procedures that take into account the specific trait being introduced will help to insure that impacts of gene flow remain low. Where trait characteristics warrant, a variety of production practices can be used to mitigate gene flow, and novel genetic/molecular containment technologies are being developed to accomplish similar goals.

The economic consequences of gene flow from biotech crops may differ in crops produced for seed (to be planted) vs. crops produced for commodity uses (to be consumed or woven into textiles), or in traditional vs. niche marketplaces. Approaches to minimize potential negative impacts are discussed.

Potential risks and benefits of maintaining or altering the existing safety and regulatory mechanisms are addressed in the context of public policy considerations. These considerations include the potential benefits of establishing thresholds for unapproved biotech substances in any commodity and for approved biotech substances in a commodity labeled as nonbiotech. Existing regulations are costly and can discourage development of beneficial products. Regulatory approaches that consider benefits and costs more holistically may facilitate improved development of these technologies.

To date, there have been no major health or environmental setbacks due to gene flow from biotech crops; in fact, these crops have led to significant, documentable improvements and, in some instances, decreased environmental risks. Education addressing the realistic advantages and challenges of continued development and commercialization of biotech crops, as well as nonbiotech crops, will be a key to public understanding and discourse related to future policy toward biotech crops.

POLLEN FLOW IN THE ENVIRONMENT - DEVELOPMENT OF A RESEARCH PROGRAM. John A. **Glaser**, Biotechnology Research Program Leader, USEPA, Office of Research & Development, National Risk Management Research Laboratory, Cincinnati, OH 45268.

The USEPA Office of Research and Development seeks to provide to the agency and society the best information relating to the status of the environment and any related technology maintaining environmental quality. In this effort, a recent research workshop (Pollen Mediated Gene Flow in the Environment) was conducted to assess the level of knowledge and possible future research directions related to pollen flow of transgenic crops. In the development of future research directions, several questions are needed to focus the inquiry. What are the important technical issues? How should the selection or prioritization of research goals be developed? What are the best research questions for field scale experimentation? Thinking of potential uses of this research, can we assemble decision making aids for transgenic crop pollen effect evaluation?

SEED-MEDIATED GENE FLOW IN CANOLA. Linda M. **Hall**, Robert H. Gulden and Hugh J. Beckie, Research Scientist/Adjunct Professor, Alberta Agriculture and Food, University of Alberta, Edmonton, AB, T6G 2P5; Assistant Professor, Department of Plant Science, University of Manitoba, Winnipeg, MB, R3T 2N2; and Research Scientist, Agriculture and Agri-Food Canada, Saskatoon, SK, S7N 0X2.

Canola or oilseed rape (*Brassica napus* L.) is a relatively newly domesticated oilseed crop grown in Canada and in temperate regions around the world. Canola resistant to glyphosate, glufosinate or imidazolinone (IM) herbicides was introduced in Canada in 1996 and has been widely adopted, occupying 45, 42 and 10% of canola acres, respectively. Herbicide-resistant canola is planted on 97% of acres but seed sources are not necessarily pure. In a study of admixture in certified canola seed, the permitted threshold of 0.25% was frequently exceeded. Harvest loss in canola due to pod shatter prior to and during harvest averaged 5.9% of the seed yield, or approximately 3,000 viable seeds m^{-2}, however, harvest losses ranged from 9 to 56 times the normal seeding rate of canola. Canola can develop secondary seed dormancy that varies with genotype, and is induced most effectively by low water potential in combination with warm temperatures. Fall tillage promotes the persistence of high dormancy genotypes. While the majority of canola volunteers are recruited in the year following dispersal, seed banks can persist for 3 to 4 years. Seed bank deletion occurs by predation, pathogenesis and desiccation, in addition to germination in spring and fall. High densities of seed can germinate pre-seeding and within a crop. Canola can be a competitive weed within cropping systems, and weed surveys have ranked it as the 14th most abundant weed in the 2000's, occurring in 11% of fields surveyed. Volunteer control options exist for all herbicide-resistant biotypes. However, with naive herbicide rotations, resistant canola can be difficult to control - for example if glyphosate is used alone pre-seeding on glyphosate-resistant canola, or if ALS inhibitors are used alone in-crop to control IM resistant canola. Seed losses along transport corridors have led to feral herbicide-resistant populations along roadsides and rail lines in Canada and Japan. These populations may persist and spread, and contribute to gene flow. Seed-mediated gene flow is a significant temporal and spatial factor in the spread of herbicide-resistant genes locally and internationally.

TIER 1 EVALUATION OF CROSSABILITY BETWEEN TRITICALE (X *TRITICOSECALE* WITTMACK) AND COMMON WHEAT, DURUM WHEAT AND RYE. Linda M. **Hall**, Melissa J. Hills, Francois Eudes, Research Scientist and Adjunct Professor, Alberta Agriculture and Food/University of Alberta, 410Ag/ForBuilding, Edmonton, AB, T6G 2P5, Instructor, Grant MacEwan, 10700-104 Avenue, Edmonton, AB T5J 4S2, Research Scientist, Alberta Agriculture and Food, Lethbridge, AB.

Development of transgenic triticale as a platform for novel bio-industrial products is predicated on an environmental biosafety assessment that quantifies the potential risks associated with its release. Pollen-mediated gene flow to related species and conventional triticale varieties is one pathway for transgene movement. A tier 1 quantification of triticale hybridization was conducted by emasculating and hand pollinating flowers under greenhouse conditions. Approximately 2000 manual pollinations were conducted for each cross and its reciprocal between two triticale genotypes: a modern triticale cultivar (AC Alta) and primary triticale (89TT108), and common wheat, durum wheat and rye. The frequency of outcrossing, hybrid seed appearance and weight, and F_1 emergence and fertility were recorded. Outcrossing, F_1 emergence and fertility rates were high from crosses between triticale genotypes. Outcrossing in inter-specific crosses was influenced by the species, and the genotype and gender of the triticale parent. In crosses to common and durum wheat where triticale was the male parent, outcrossing was $\geq$73.0% and $\geq$69.5%, respectively, but $\leq$23.9% and $\leq$3.0% when triticale was the female parent. Overall, outcrossing with rye was lower than with common and durum wheat. F_1 hybrid emergence was greater when triticale was the female parent. With the exception of a single seed, all wheat-triticale F_1 hybrid seeds were non-viable when triticale was the male parent in the cross. Only 7 durum wheat-triticale F_1 hybrids emerged from 163 seeds sown and all were produced with triticale 89TT108 as female parent. With rye, 8 F_1 hybrids emerged from 38 seeds sown and all were produced from crosses to AC Alta; 5 with AC Alta as the female parent and 3 as the male. Interspecific F_1 hybrids were self-sterile, with the exception of those produced in crosses between common wheat and triticale where triticale was the female parent. Tier 2 hybridization quantification will be conducted under field conditions.

GENE FLOW DYNAMICS AND CONFINEMENT: A REGULATORY PERSPECTIVE. Subray **Hegde***, Biotechnology Regulatory Services/Animal and Plant Health Inspection Service, United States Department of Agriculture, Riverdale, MD.

Gene flow is a natural biological process with potential evolutionary consequences. For a few human activities, however, gene flow from certain source populations into the environment is undesirable, which prompted the development of confinement protocols to contain gene flow to a defined physical space. A variety of confinement protocols are currently in use to prevent unintended gene flow from genetically engineered (GE) crops beginning from their creations to their intended use. Because gene escape from focal populations can occur in time and space, and is affected by a variety of genetic and ecological factors, the existing confinement protocols have gradually been evolving to address new issues and concerns raised by stakeholders. A few issues that could significantly alter the confinement principle in the future are: (i) an acceptable level of gene flow from GE plant populations into the environment, (ii) the cost and benefit of doing business with alternate gene flow containment strategies, and (iii) the public perception about genetically modified plants and plant products.

GENE FLOW AND RISK ASSESSMENT: CASE BY CASE CONSIDERATIONS. Michael J. **Horak** and Thomas E. Nickson, Monsanto Company, St. Louis, MO.

A fundamental tenant of risk assessment is that risk is a function of *hazard* and *exposure*. For biotechnology-derived crops, a risk assessor considers potential *hazards* associated with the crop that could include altered pest potential and potential adverse environmental impacts including adverse effects on non-target organisms. A risk assessor also considers e*xposure* by examining reasonable pathways for release into the environment, the environmental fate of the plant and trait, and potential routes of exposure to non-target organisms. The assessor would also evaluate information on potential gene flow via pollen, seed and tissue, and information on potential consequences of gene flow. The hazard and exposure information is then incorporated into the overall risk assessment.

During the risk assessment planning phase, the nature of the crop, the nature of the trait, the likely receiving, and the interactions among these factors are considered to identify potential hazards. Then comparative plant characterization data are generated on the biotechnology-derived crop. The comparative data are assessed for unintended and/or potentially adverse differences in the plant, and trait advantages to the crop that potentially affect the weediness of the crop. This information is used in an assessment of potential hazards of the crop. Concurrently, data are generated for an assessment of the potential effects to non-target organisms. The data from the hazard characterization are then considered in the context of a gene flow assessment (a portion of the exposure assessment). The gene flow assessment considers gene flow within the crop species and the likelihood of trait introgression into a sexually compatible species. Together the information from the hazard assessment and the exposure assessment, including gene flow information, are used in an overall assessment of risk.

BIOSAFETY ASSESSMENT AND BENEFITS FOR CO-EXISTENCE OF BIOLOGICAL CONTAINED PLANTS – REGULATORY ASSESSMENT IN THE EU-PROJECT "TRANSCONTAINER". Christiane **Koziolek** and Detlef Bartsch, Professor, Federal Office of Food Safety and Consumer Protection, Mauerstrasse 39-42, D-Berlin 10117.

The EU-project TransContainer[1] deals with the evaluation of environmental impact and benefits for coexistence between GM- and non-GM plants. Different containment strategies are applied to a broad spectrum of crops (*e.g.* oilseed rape, sugar beet, tomato) as well as to perennial plants like trees and grasses. The containment methods focus on three strategies: Chloroplast Transformation, Controllable Flowering and Controllable Fertility. Key issues are (a) safety assessment focussing on three points: molecular characteristics, ecology of the GM species and consequences of a potential break-down of the containment system. (b) Benefit assessment of the containment system for the co-existence of GM and non-GM plants. The environmental impact assessment is performed based on the criteria provided by the EFSA Guidance document (2006)[2] for the placing on the market of GM plants in the EU. Additionally to impact assessment, an economic evaluation is performed. The development of contained GM crop plants is still in an early stage and thus our evaluation is focussed on the safety assessment of the general methodical characteristics.

Benefits of Chloroplast Transformed plants: Chloroplast transformation is a promising containment system for plant species with strict maternal plastid inheritance by avoiding the out-crossing of recombinant genes via the pollen. The targeted insertion of a GM sequence in the chloroplast genome by homologous recombination has three advantages: (1) insertional inactivation of unknown functional genes is avoided, (2) endogenic *in-situ* promoters can be used, and (3) even though plastids harbour relatively small replicons, large insertions are tolerated. However, it has to be verified that the transgene is not inserted unintentionally in the nuclear genome since the transformation (*e.g.* particle bombardment technique) could be unspecific.

Benefits of plants with Controlled Flowering: The suppression of flowering is useful for plants that are cultivated for their vegetative parts, *e.g.* sugar beet, grasses or trees. For bi-annual sugar beet, the inhibition of undesired bolting and flowering will either prevent out-crossing and introgression into endogenous beet populations as well as facilitate the cultivation of beets for the farmer. In grasses, flower suppression will improve the fodder quality as the shoots have higher lignin content thus hampering the digestibility of the feed. In trees that are intended to be cultivated in plantations for biomass production, the suppression of flower development would offer certain advantages: GM trees with *e.g.* changed wood properties would neither develop pollen nor seeds containing the inserted DNA, avoiding any unintended hybridisation or spread of the GM seeds by wind. In contrast to trees that can be propagated vegetative, grasses as well as sugar beet need to flower for breeding purposes. Therefore, a molecular switch will be introduced, which will initiate flowering upon an external chemical stimulus. In case of flower induction for breeding, other (physical) measures are requested to avoid unintended out-crossing and spread of hybrids.

Benefits for Co-existence: Regarding the legislation on GM plants in the EU, the minimisation of GM escape via pollen into adjacent non-GM fields or wild relatives is an important point in the improvement of co-existence measures. In chloroplast transformed plants, the spread of inserted DNA is limited only at the pollen level, whereas the (transgene) seeds produced by the mother plant could still be lost or spilled unintended during harvest and transport. Upon complete flower suppression, neither pollen nor seeds will be released, thus gaining a very high level of restriction. The containment measures will allow (i) minimising the isolation distances between GM and non-GM fields, (ii) protecting the GM cultivating farmers for liability claims from neighbours, (iii) reduce conflicts with bee keepers, and (iv) avoid unintended mixtures with food products.

VARIATION IN ALS HERBICIDE RESISTANCE OF DIVERSE CROP-WILD SUNFLOWER HYBRIDS.

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Across the range of sunflower production, there is considerable gene flow from crop fields into to wild sunflower (*Helianthus annuus* L.) populations. With the development of herbicide resistant crops, concerns have surfaced about the transfer of herbicide resistance to wild relatives. Even with stewardship guidelines associated with herbicide resistant varieties, which require farmers to control wild sunflower populations in and around their fields, we expect to see gene flow into wild populations. We performed greenhouse and field experiments to understand the role of genetic variation, both within and among wild sunflower populations, in determining the herbicide tolerance of the crop-wild hybrids they produce. We created crop-wild hybrids by crossing diverse wild populations with pollen from either ALS resistant crop or conventional crop sunflower. We tested the resistance of these crop-wild hybrids and their wild counterparts to Pursuit, an imidazolinone herbicide, and Express, a sulfonylurea herbicide. Field grown crop-wild hybrids with putative ALS resistance were more tolerant of the herbicide applications than their susceptible wild and hybrid counterparts, evidenced by taller seedlings with more leaves, and less injury to their primary meristem. However, that advantage did not last through the season. Compensatory growth resulted in greater seed production by surviving non-herbicide resistant hybrids and wilds. Follow-up experiments in the greenhouse indicated that both the origin of the wild population and the family within a population affected herbicide resistance expressed in crop-wild hybrids. Therefore, the adaptive advantage conferred by the herbicide resistance will vary depending on the wild genetic background and may not last throughout the season unless other stressors are applied.

MEASURING THE EFFECTS OF CROP GENETIC LOAD ON PRODUCTIVITY AND FITNESS IN WEEDY *BRASSICA RAPA* (WILD TURNIP) × *BRASSICA NAPUS* (OILSEED RAPE) HYBRID POPULATIONS. Reginald J. **Millwood***, Christy W. Rose*, and C. Neal Stewart, Jr. University of Tennessee, Knoxville 37966.

With the implementation of transgenic crops in agriculture, transgene flow to wild relatives is sure to occur. In the event transgenic hybrids are produced, the inherited transgene could supply a fitness advantage. This is a real agronomic and ecological concern, but only if transgene introgression occurs. In many cases, hybrids such as these exhibit lower fitness when compared to their wild parents. This may be due to the inheritance of disadvantageous crop/domestication genes present in the new host genome. These genes would certainly negatively impact transgene introgression and possible reduce the risk associated with many transgenes. In order to gain a better understanding of transgene introgression, we plan to assess how productivity and fitness of backcrossed hybrids are affected by the presence of a transgene in the company of crop/domestication alleles. Here, we use transgenic *Brassica napus* cv. westar as a model crop plant. *B. napus* has been transformed with the *Bacillus thuringiensis* endotoxin (*BtCry1Ac*) and the green fluorescent protein. Subsequently, we made hybrids between the transgenic *B. napus* and its wild weedy relative *Brassica rapa* ac. 2974. We produced mixed BC_1/F_2 populations in the field as well as advanced backcross generations (F_1, BC_1F_1, BC_2F_1 BC_2F_2, BC_3F_1 and BC_4F_2) by hand-crossing in controlled environment chambers. First, we plan to grow the BC_1/F_2 populations in competition with *Triticum aestivum* as well as with each other. Productivity and fitness data will be gathered for both the hybrids and wheat. Secondly, we will grow the advanced backcross populations under agronomic conditions. Productivity data such as seed yield and dry above ground biomass will be recorded. In addition, to gain an understanding of hybrid fitness, we will also grow these plants in competition with *B. rapa* and the number of transgenic progeny will be recorded. All of the above data will then be correlated to the amount of crop specific AFLP markers present in each population. These data together will determine if there is any relationship between inherited crop/domestication alleles and productivity or fitness of transgenic hybrids of *B. rapa* × *B. napus*.

STRATEGIES TO REDUCE TRANSGENE MOVEMENT Hong S. **Moon**, Jason N. Burris, Reginald J. Millwood and C. Neal Stewart, Jr., Department of Plant Sciences, The University of Tennessee, Knoxville, TN 37996

Transgene escape is of a major ecological concern when growing transgenic plants in the field. To address these concerns, suitable strategies for transgene containment must be created. Currently, two strategies that can be utilized as transgene containment and control are male sterility and site-specific recombination. First, male sterility can be obtained by making interspecific hybrids of *Nicotiana tabacum* X *Nicotiana glaca*. By using the genetic “distance” and a large difference in chromosome number, we can produce non-functional gametes, and thus, create functional sterility. We will transform male sterile hybrids with fluorescent-protein markers to track the potential of pollen formed in the field. Secondly, a transgene excision system using a site-specific recombinase or a zinc finger nuclease will be created in order to remove transgenes from the pollen. A model plant, canola or tobacco, will be transformed via *Agrobacterium*-mediated methods with constructs containing site-specific recombinases or zinc finger nucleases. This system will employ a visual marker green fluorescence protein driven by pollen-specific promoter to ensure transgene excision. Pollen-specific promoters, *LAT52* and *LAT59*, will be used to activate the recombinase or zinc finger nuclease in pollen to induce the excision of transgenes.

WEED-TO-WEED GENE FLOW – WHAT IS THE POTENTIAL FOR GLYHPOSATE RESISTANCE MOVEMENT VIA INTERSPECIFIC HBRIDIZATION? Micheal D.K. **Owen** and Ian A. Zelaya, Professor, Agronomy Department, Iowa State University, Ames, IA 50011-1011 and Project Team Leader, Syngenta Ltd., Weed Control Research, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, United Kingdom

When evaluating herbicide resistance and the potential for herbicide resistance dissemination within agroecosystems, most studies focus on weed seed production, viability and dispersal, the species mating system, and the genetics associated with the resistance trait. However, far less attention has been centered on the potential for herbicide resistance spread though interspecific hybridization (introgression). For that matter, the ability of weeds to hybridize with near relatives has not been well-studied or documented in agro-ecosystems, however some data exists for Amaranthaceae. While some studies describe gene flow and successful transmission of herbicide resistance alleles from cultivated crop species to their weedy relatives, little data exists regarding the between weed species gene flow. Part of the problem describing weed hybridization is recognition of the hybrids in the field; this is particularly challenging since hybrids in most cases resemble (phenotypically) the parents.

Herbicide resistance potentially represents an effective marker to document hybridization between weed species. This study assessed the introgression of herbicide resistant alleles in the important genii *Ambrosia*, *Conyza* and *Helianthus*, considering the importance of Asteraceae weeds in current agroecosystems. The study used the cross resistant triazolopyrimidine, sulfonanilide and sulfonylurea (SU) giant ragweed (*A. trifida* L.; $2n = 24$) and imidazolinone and SU common sunflower (*H. annuus* L.; $2n = 34$) populations from Iowa, in addition to a glyphosate resistant horseweed (*C. canadensis* L. Cronq.; $2n = 18$) population from Delaware. The herbicide susceptible common ragweed (*A. artemisiifolia* L.; $2n = 36$), Jerusalem artichoke (*H. tuberous* L.; $2n = 102$), and dwarf fleabane (*C. ramosissima* Cronq.; $2n = 18$) populations originated from Iowa. In all three genii evaluated, herbicide resistance was expressed as a nuclear allele, partially dominant (*Ambrosia* and *Helianthus*) or over dominant (*Conyza*) trait in first filial interspecific hybrid generation (F_1^H). Herbicide resistance transfer frequency in the F_1^H ranged from 40 to 60%, 0 to 9%, and 30 to 50% in *Ambrosia*, *Conyza*, and *Helianthus*, respectively.

Inheritance of herbicide resistance in the selfed F_1^H generation (F_2^H) followed the mono-factorial model in all evaluated genii; F_1^H backcrosses confirmed successful introgression of the herbicide resistance allele to parents. Thus, interspecific hybridization is possible and may be a potentially important avenue for the dissemination of herbicide resistance, specifically glyphosate resistance, and the promotion of genetic diversity within compatible weed species.

The occurrence of glyphosate resistant hybrid weeds may complicate the containment of glyphosate resistance in current crop production systems. However, it has yet to be determined if interspecific hybridization of weed species is frequent and pervasive within the agro-ecosystems. If the occurrence of weed hybridization is relatively frequent, important ecological studies should be conducted to determine if the weedy hybrids are well-adapted to current crop production systems and thus may evolve into economically important weed problems. The movement of glyphosate resistance via gene flow as a companion to the hybridization process could improve the adaptation of weedy hybrid progeny to the agro-ecosystem. Despite the likely negative implications of evolved glyphosate resistance and weed hybridization , the occurrence of glyphosate resistance could be a useful marker to assess weed hybridization.

ADDRESSING GENE FLOW ISSUES IN COWPEA FOR WEST AFRICA.

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Cowpea (*Vigna unguiculata*), known in the U.S. as "black-eyed pea," is a legume crop grown and consumed across West Africa. Native to East Africa, cowpeas are relatively drought resistant and the grain serves as an important source of quality protein. Like the grain, the leaves are rich in protein and are consumed in salads and used as animal fodder. Insects, particularly pod boring insects, can reduce yields as much as 90% or more. One of the primary limiting pests is the legume pod borer (*Maruca vitrata*), a lepidopteran insect closely related to the European corn borer, which has been successfully controlled by several different *Bacillus thuringiensis* (*Bt*) genes. An international group of scientists organized as the Network for the Genetic Improvement of Cowpea for Africa (NGICA) in cooperation with the African Agricultural Technology Foundation (AATF) is developing a transgenic cowpea variety engineered to express an insecticidal *Bacillus thuringiensis* (*Bt*) Cry1Ab protein to control this pest. Key issues to be addressed are gene flow potential and weediness particularly within areas of cultivation. To that end, assessments are under way to determine 1) outcrossing rates, 2) outcrossing distances, 3) the potential for insects to vector pollen, and 4) the consequences of gene flow as it relates to the fitness potential (insect resistance) of wild or weedy species. These data will be used in conjunction with cultivated cowpea production data to assess potential environmental effects of *Bt*-cowpea in West Africa.

ECOLOGICAL EFFECTS OF VIRUS-RESISTANT TRANSGENIC SQUASH ON WILD SQUASH POPULATION DYNAMICS.

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Several genetic and environmental factors can influence the degree of assortative mating in natural plant populations. In many, perhaps most, natural plant populations assortative mating occurs because plants do not have identical flowering schedules. When only one phenotype is in bloom, mating is necessarily assortative, leading to increased genetic variance for flowering phenology. This is important because increased genetic variance allows a trait to be more responsive to natural selection. Thus, fixation of a trait favored by selection will be faster when a population mates assortatively. For example, if the presence of a transgene in a wild population leads to assortative mating, then the transgene will introgress into that population more rapidly than expected. In a common garden experiment healthy and virus-infected squash had different flowering phenologies. These differences in flowering phenology will lead to assortative mating among virus susceptible and virus resistant plants. In another set of common garden experiments we found that bumbles bees spent more time in flowers on virus infected plants, while squash bees spent more time in flowers on healthy plants. These data suggest that if a transgene for virus resistance were present in a wild population it could lead to assortative mating, and thus increase in frequency more rapidly than anticipated. In contrast to differences in flowering phenology and pollinator preference, which cause assortative mating, temporal variation in sex ratios results in disassortative mating. Thus, different flowering phenologies and pollinator preferences will lead to assortative mating and increased genetic variance, while temporal variation in sex ratios will lead to disassortative mating and reduced genetic variance. Interactions between these processes and their effect on character evolution (e.g. transgenic virus- resistance) are under investigation.

INTERLOPER'S LEGACY: INVASIVE, HYBRID-DERIVED CALIFORNIA WILD RADISH (*RAPHANUS SATIVUS*) EVOLVES TO OUTPERFORM ITS IMMIGRANT PARENTS. Caroline E. **Ridley**, Rosamond F. Tsao and Norman C. Ellstrand, Graduate Student, Undergraduate Student and Professor of Genetics, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.

Hybridization between species and subspecies may lead to the evolution of invasive weeds by enhancing survival and reproduction in hybrid-derived lineages. California wild radish (*Raphanus sativus* × *Raphanus raphanistrum*) is a hybrid-derived species that has spread prolifically within the last 150 years, replacing all pure parental populations throughout California. Though highly plausible, a link between hybridization and invasiveness in California wild radish has never been empirically tested. In field experiments, we compared the survival and reproduction of several populations of California wild radish with that of populations of its pure parents in multiple years and varied environments. California wild radish has high survivorship and generally produces more fruits per plant, more seeds per fruit and more seeds per plant than either of its progenitors. In year one in Riverside, CA, it produced 3-times more seeds per plant than *R. raphanistrum* and *R. sativus*. In Irvine, CA, reproduction was higher overall and California wild radish produced 2-times and 20-times more seeds per plant than *R. raphanistrum* and *R. sativus*, respectively. Individual populations of California wild radish also display a strong genotype-by-environment interaction, indicating genetic diversity may be partly responsible for the weed's ability to invade California's vast and varied landscape. Our results demonstrate that by limiting the introduction and subsequent hybridization of congeners, we may be able to prevent the evolution of new invasive lineages.

SYMPATRY AND HYBRIDIZATION OF CANOLA AND BIRD RAPE (*BRASSICA RAPA* L.) IN QUÉBEC. Marie-Josée **Simard**, Anne Légère, and Suzanne I. Warwick, Research Scientist, Agriculture and Agri-Food Canada (AAFC), Québec, QC G1V 2J3; Research Scientist, AAFC, Saskatoon, SK S7N 0X2, Research Scientist, AAFC, Ottawa, ON K1A 0C6.

Hybridization between herbicide resistant (HR) transgenic canola (*Brassica napus* L.) and weedy bird rape (*B. rapa* L., also birdsrape mustard) has been documented in Québec. We evaluated the actual hybridization potential based on range overlap and *in situ* rates. We mapped the distribution of canola fields and bird rape herbarium specimens in Québec; collated information on the presence of bird rape in certified canola seed production fields; and surveyed for bird rape in, or close to canola field margins. Progeny from these populations was screened for herbicide resistance (HR) and for the presence of the HR transgene. Significant sympatry was observed in several areas and hybridization occurred in all eight populations (1.1-17.5% hybrid seed) located in field margins and in one (1.1%) out of three populations located less than 10 m from a canola field. Hybridization rates decreased exponentially as bird rape density increased, but rates across plants at any given density were highly variable (0 to 68%). At present, there are no compelling data suggesting that the presence of an HR transgene in a wild/weedy relative is inherently risky. However, our current knowledge might not fully describe the risks posed by other transgenes, particularly those that convey fitness-enhancing traits.

LONG-TERM FIELD STUDIES OF THE EVOLUTION OF CROP-WEED HYBRIDS IN RADISH: IMPLICATIONS FOR INVASIVENESS. Allison A. **Snow**, Lesley G. Campbell, Theresa M. Culley, and Caroline E. Ridley, Professor, Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH 43210, Postdoctoral Associate, Departments of Plant Science, Landscape Architecture, and Entomology, University of Maryland, College Park, MD 20742, Assistant Professor, Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221,

and Graduate Student, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.

Many cultivated plants hybridize naturally with wild and weedy relatives, but little is known about the evolutionary effects of this process on recipient populations. To examine the dynamics of introgression in a natural setting, we monitored crop-specific genetic markers in replicated field populations of weedy *Raphanus raphanistrum* in Michigan, USA, for ten years. Four isolated hybrid populations were established in 1996 using a 1:1 ratio of *R. raphanistrum* and F_1 crop-wild hybrids (*R. raphanistrum* x *R. sativus*). The sites were tilled and fertilized annually to mimic agricultural fields, and plants were exposed to local biotic and abiotic selective pressures. Initially, F_1 hybrids had reduced fitness relative to wild genotypes, but the populations quickly regained wild-type pollen fertility, presumably by losing a crop-specific reciprocal translocation. Recombination and natural selection allowed the populations to absorb two crop-specific allozyme markers at relatively high frequencies in all populations, even exceeding their initial frequency of 0.25 in a few cases. Frequencies of a crop-specific white petal color allele were much lower, but this allele also persisted in all populations. Overall, frequencies of the three crop-specific alleles varied considerably among locations, years, and loci. In the tenth year, plants from each hybrid population were grown in a common garden experiment along with wild genotypes. The lifetime fecundity of these advanced-generation hybrids was similar to that of the wild genotypes. This long-term study provides a unique example of how easily certain crop alleles can become established in weed populations while others remain rare or disappear.

In a second study, we tested the hypothesis that crop-wild hybridization can allow weeds to become more successful. A third study was carried out simultaneously to determine whether cultivated radish could generate feral populations. We established replicated populations of wild, hybrid, and "volunteer" cultivated radishes in Michigan and let them evolve for three growing seasons, starting in 2002. Three of the five volunteer populations died out. The two remaining populations became contaminated with wild genes and evolved traits that were similar to crop-wild hybrids (Campbell and Snow, in prep.). Although we did not find evidence for ferality in the absence of hybridization with *R. raphanistrum*, further studies involving more populations and locations might detect ferality.

Results from the wild and hybrid populations were reported in Campbell et al. (2006) and are summarized briefly here. The initial frequency of crop alleles in these hybrid populations was 0.50 (twice the level in our introgression study above) because all plants were F_1 hybrids. Frequencies of white-flowered plants declined slightly, unlike our previous study, and then remained relatively constant. We suspect that the sharp drop in white-flowered plants in our previous study was due to the fact that many hybrid plants flowered very late or not at all, and the white petal allele is linked to delayed reproduction (Campbell, 2007). In 2005, advanced-generation hybrid and wild seedlings were grown in common garden experiments in Michigan and California. Hybrid-derived plants had slightly lower fecundity than wild plants in Michigan, but exhibited ~270% greater lifetime fecundity and ~22% greater survival than wild plants in California. These results support that hypothesis that crop-wild hybridization may create genotypes with the potential to displace parental taxa in new environments, which is consistent with other studies of hybrid-derived wild radish populations in California (C. E. Ridley et al., in prep.). In summary, our combined field studies of evolving crop-wild hybrids show that conventional crop alleles can persist in wild populations and may increase the fitness of wild relatives in some cases. Further research is needed to confirm the common assumption that enhanced fitness results in more abundant weed populations.

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LIFETIME FECUNDITY OF F1 CROP-WILD SORGHUM HYBRIDS: IMPLICATIONS FOR GENE FLOW FROM TRANSGENIC SORGHUM IN AFRICA. Allison A. **Snow**, Patricia M. Sweeney*, Cécile Grenier, Gebisa Ejeta, Tesfaye Tesso, Issoufrou Kapran, Gurling Bothma, and Jeffrey F. Pedersen, Professor and Research Associate, respectively, Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, OH, 43210; Research Geneticist and Professor, respectively, Department of Agronomy, Purdue University, West Lafayette, IN, 47907; Researcher and Leader, National Sorghum Research Project, Ethiopian Institute of Agricultural Research, Melkassa Research Center, Nazareth, Ethiopia; Principle Sorghum Breeder, Institut National de la Recherche Agronomique du Niger, Niamey, République du Niger; Researcher, Agricultural Research Council-Roodeplaat, Pretoria, South Africa; and Research Geneticist, United States Department of Agriculture-Agriculture Research Service, University of Nebraska, Lincoln, NE, 68583.

Researchers are developing transgenic crops with enhanced nutrition and higher yields for Africa, but few studies have assessed environmental risks of growing these crops. Since wild relatives of sorghum (*Sorghum bicolor*) are often weedy and represent valuable germplasm, plans to release transgenic sorghum should consider consequences of gene flow. Our previous studies in Ethiopia and Niger showed that wild and cultivated sorghum often co-occur and flower simultaneously. Here, we tested for spontaneous hybridization between accessions of wild *S. bicolor* and local cultivars from eastern Africa at times when their flowering overlapped. Plants were grown in field plots in Ohio, with a ratio of more than 20 crop plants per wild individual. Microsatellite DNA markers showed that some seeds on wild plants were fertilized by crop pollen. We also studied the fecundity of F_1 hybrids between a male-sterile cultivar and three wild accessions. Wild and hybrid progeny were grown in Niger, Ohio, and Indiana. The relative fecundity of hybrids was fairly consistent across locations but differed somewhat among accessions. For two accessions, crop-wild hybrids produced more seeds per plant than their wild parent. For a third accession, hybrids produced similar numbers of seeds per plant in Niger, but fewer seeds per plant in the USA. However, this decrease in seed per plant in the USA was not significant. Although one hybrid had poor seedling survival, once established, all crop-wild F_1 hybrids were vigorous, and fertile, and could easily contribute pollen and seeds to subsequent generations. This study shows that selectively neutral or advantageous crop alleles are likely to persist in wild sorghum populations following hybridization. Before transgenic sorghum varieties are grown near wild relatives in Africa, ecological effects and other consequences of crop-to-wild gene flow should be examined for each transgenic trait.

SEED-TO-SEED AND HAY-TO-SEED POLLEN MEDIATED GENE FLOW IN ALFALFA. Larry R. **Teuber***, Shannon Mueller, Allen Van Deynze, Sharie Fitzpatrick, James R. Hagler, and Jose Arias, University of California, Davis 95616, Forage Genetics, Inc, West Salem, WI 54669, and ARS-USDA, Arid-Land Agricultural Research Center, Maricopa, AZ 85239.

Honey bees (*Apis mellifera* L.) are predominantly used in California as pollinators for alfalfa (*Medicago sativa* L.) seed production. In some areas there is an increasing use of leafcutter bees (*Megachile rotundata* Fabricius.) in combination with honey bees. It is well known that honey bees will forage up to several miles from their hive. A study conducted in 2003 by our group conducted a gene flow study with a 6 acre Roundup Ready® source plot and eleven 0.54A trap plots at regular intervals extending East and West of the source. That study demonstrated adventitious presence (AP) in excess of 1.5% 900 ft from the marker gene source plot. Furthermore, the marker gene was detectable at very low frequency out to 2.5 miles – the outer limit of the study. The objectives of the current studies were to 1) evaluate the effectiveness of commercially available test kits in detecting the presence of the CP4 EPSPS (Roundup Ready) protein in seed samples from the 2003 study and known to have low levels of the trait based on extensive seedling growouts, 2) to determine the degree to which genes present in alfalfa fields being produced for hay are transferred to adjacent seed fields located the minimal legal distance of 165 ft from the hay field, and 3) to study gene flow between commercial scale production fields to further determine the extent of potential gene flow between alfalfa cultivars within the foraging range of honey bees.

To assess the effectiveness of the Roundup Ready test strips for seed we used seed produced on each of the 0.54 A trap plots during the 2003 study. All evaluations we conduced in accordance with the manufacturer's instructions. A total of 125 test strips were used to determine if the CP4 EPSPS protein was present in each of the traps. Strip test results from traps with a percentage AP less than 1% as determined by seedling growouts of seventy- to ninety-thousand seedlings provided virtually identical AP percentages based on determinations using “Seed Calc”. AP percentages approaching and in excess of 1% could not be quantified because the frequency of AP seeds caused all the test strips to give positive results. For research purposes, we reduced the number of seeds tested when we started getting all positive strips, but kept all the other procedures the same. Results with this modification have also been in agreement with AP percentages found in large scale seedling growouts.

Conventional seed production fields were planted radiating out from a Roundup Ready hay production field. On all sides of the hay field the seed field was planted to within 165 ft and pollinated using honey bees. During the pollination period of approximately 8 weeks, the hay field was allowed to develop approximately 20% bloom (at least one open flower on 20% of the stems in the field) prior to being cut for hay. This is an amount of bloom the will occur with some commercial hay production and results in an opportunity for bees to visit the flowers and tripping does occur. Under this protocol, however, no seed is produced in the hay field. This degree of bloom was allowed to occurred in two consecutive cutting cycles during pollination. Seed was harvested at maturity from the seed fields at 50 foot intervals between 165 ft from the hay field (0 to 3 ft into the seed field) out to 615 ft from the hay field. Based only on test strip assessment, AP percentage was 0.29 % at 165 ft and dropped to less than 0.1% within 200 feet (365 ft of the hay field). This percentage of AP is well within current standards for varietal purity in the Federal Seed Law.

Seed to seed gene flow was studied in commercial seed production fields in the San Joaquin Valley of California. The source field was a 240A planted to cultivar bred to express the CP4 EPSPS protein. This field was isolated from all other seed production, except fields within the study area, by three miles in all directions. Within in the study area, a conventional cultivars was being produced for seed at 1 mile (240A), 3 miles (40A), and 5 miles (100A). All commercial seed production was pollinated by a combination of honey bees and leafcutter bees. 1.8A bridged trap plots were located on one edge of the study at 900 ft intervals between the source field and the conventional cultivar located 1 mile away. The first of these traps was located 165 ft from the source field. Current results are preliminary and are based on test strips. Equal size (1.8A) study areas were intensely sampled within each of the commercial. Among the small bridged traps, AP averaged 2.3% at 165 ft and rapidly decreased to 0.9% at 900 feet and 0.6% at approximately 4000 ft. At one mile AP percentage was less than 0.2%. At three miles the AP percentage was less than 0.03%. AP was not detected 5 miles from the source plot. Growouts of seedlings from form these test areas are still in progress. However, current data from this study using strip tests is in very close agreement with seedling growout data from our previous study.

APPLICATION OF LARGE EDDY SIMULATION TO QUANTIFY DISPERSAL OF VIABLE MAIZE POLLEN. Brian **Viner**, Ray Arritt, Mark Westgate and Susana Goggi. Graduate Research Assistant, Professor, and Professor, Department of Agronomy, Assistant Professor, Department of Seed Science, Iowa State University, Ames, IA 50011.

The creation of genetically modified (GM) crops has raised concerns regarding the transfer of genes from GM crops to wild relatives. To assess the risk of outcross, the development of numerical models that can accurately predict the movement depositional viability of pollen is needed. In maize, the

primary mode of pollination is the transport of pollen by wind. Large Eddy Simulation (LES) is a tool to model turbulent motions that have the potential to lift pollen high into the atmospheric boundary layer and transport it over distances of at least five kilometers.

A LES model has been combined with a Lagrangian Dispersion Model to predict the transport and viability of pollen in the atmosphere. Predictions have been made for the deposition of maize pollen and of pollen viability upon deposition, as well as for vertical profiles of concentration and viability through the boundary layer. Viable pollen is modeled to be distributed throughout the boundary layer and transported over five kilometers before reaching the ground.

REGULATION OF DIURNAL POLLEN RELEASE IN MAIZE. Brian **Viner**, Raymond Arritt and Mark Westgate. Graduate Research Assistant, Professor and Professor, Department of Agronomy, Iowa State University, Ames, IA 50011.

The ability to accurately model pollen dispersion is reliant on reasonably predicting the magnitude of pollen shed over the day. To develop a predictive equation, pollen was collected at two field sites from 29 July to 3 Aug 2003. Based on measurements of collected pollen, a rate of pollen shed was calculated over each day and normalized to the total amount of pollen collected. Our model predicts the rate of shed as a function of two processes. The first equation is a Gaussian curve that predicts the percentage of pollen that is available for shed as a function of vapor pressure deficit. The second process predicts the amount of available pollen that will be shed. The output from this model would provide the rate of pollen shed in terms of the percent of a day's total shed. Results from this model show R^2 values ranging between 0.54 and 0.99 when compared to our field observations.

DO ESCAPED TRANSGENES PERSIST IN NATURE? THE CASE OF AN HERBICIDE RESISTANCE TRANSGENE IN WEEDY POPULATIONS OF *BRASSICA RAPA*. Suzanne I. **Warwick**, Anne Légère, Marie-Josée Simard and Tracey James, Research Scientist, Agriculture and Agri-Food Canada (AAFC), Ottawa, ON K1A OC6; Research Scientist, AAFC, Saskatoon, SK S7N 0X2; Research Scientist, AAFC, Québec, QC G1V 2J3; Technician, AAFC, Ottawa, ON K1A OC6.

This is the first report of the persistence and apparent introgression of an herbicide resistance transgene from canola (*Brassica napus)* into the gene pool of its weedy relative, bird rape (*B. rapa* L., also birdsrape mustard), monitored under natural commercial field conditions. Hybridization between glyphosate-resistant (HR) *B. napus* and *B. rapa* was first observed at two Québec sites, Ste-Agathe and St-Henri, in 2001. *Brassica rapa* populations at these two locations were monitored in 2002, 2003 and 2005 for the presence of hybrids and transgene persistence. All plants were scored for the HR trait (HR+/HR-), presence of species-specific AFLP molecular markers from both parental species, pollen viability, and ploidy level. Hybrid numbers decreased over the 3-year period, from 85 out of ca. 200 plants surveyed in 2002 to only 5 out of 200 plants in 2005 (St-Henri site). Most hybrids had the HR trait, reduced male fertility, intermediate genome structure, and presence of both species-specific AFLP markers. Both F1 and backcross hybrid generations were detected. One introgressed individual, i.e. with the HR trait and diploid ploidy level of *B. rapa*, was observed in 2005. The latter had reduced fertility but produced ca. 480 seeds. Forty-eight of the 50 progeny grown from this plant were diploid with high pollen viability and 22 had the transgene (1:1 segregation). These observations confirm the persistence of the HR trait over time. Persistence occurred over a six year period, in the absence of herbicide selection pressure (with the exception of possible exposure to glyphosate in 2002), and in spite of the fitness cost associated with hybridization.

INTER-SPECIFIC GENE FLOW IN CANOLA. Suzanne I. **Warwick**, Research Scientist, Agriculture and Agri-Food Canada (AAFC), Ottawa, ON K1A OC6.

Canola (*Brassica napus*) is capable of genetic exchange with related *Brassica* crop species as well as with several wild relatives. The large-scale use of herbicide-resistant (HR) canola has allowed us to examine inter-specific gene flow on realistic field scales. The HR trait is easy to monitor, provides accurate assessments, and is highly suited for extensive screening programs. Recent studies documenting gene flow distances of up to 200m between HR canola fields and Polish canola (*Brassica rapa*) and oriental mustard *(Brassica juncea*) fields will be presented. *Brassica napus* can potentially hybridize with four related weedy species in Canada and the United States (bird rape, *Brassica rapa*; wild radish, *Raphanus raphanistrum*; dog mustard, *Erucastrum gallicum*, and wild mustard *Sinapis arvensis*). Interspecific gene flow results with these four species will be reviewed, and will include the first report of the persistence and apparent introgression of an HR transgene from canola into the gene pool of *B. rapa*, monitored under natural commercial field conditions. Subsequent studies in eastern Canada confirm that hybridization is frequent throughout the sympatric ranges of these two species. Additional canola-quality *Brassica* crops (*B. juncea*, *B. carinata*) are under development, and interspecific gene flow concerns for these species will be reviewed and recent data from *Sinapis arvensis* x *B. juncea* hybridization studies presented. Consequences of hybridization and introgression are dependent on the traits that are introduced and their effect on hybrid fitness. The results from recent fitness trials for herbicide resistant (HR) and insect resistant Bt weed-crop hybrids (*B. rapa* x *B. napus*), suggest a cost to hybridization, independent of the transgenic trait. Future research needs will be outlined including a need for empirical data on: ecological effects of fitness-enhancing traits such as stress-tolerances, the consequences of transgene spread to non-agricultural habitats (now largely undocumented), and what specific environmental risks transgenic hybrid weed populations pose under field conditions.

MODELING THE BIOLOGY OF OUT-CROSSING BY ADVENTITIOUS POLLEN. Mark **Westgate**, Juan Astini, Agustin Fonseca, Jon Lizaso, Craig Clark, and Ray Arritt, Professor, and Graduate Student, Agronomy Department, Iowa State University, Ames, IA 50011, Research Scientist, Monsanto Company, Williamsburg, IA 52361, Crop Modeler, McNair Bostick Simulation Laboratory, University of Florida, Gainesville, FL 32611-0570, Assistant Professor, Department of Geography and Meteorology, Valparaiso University, Valparaiso, IN 46383-6493, and Professor, Agronomy Department, Iowa State University, Ames, IA 50011.

Risk of out-crossing from adventitious maize pollen results from complex interactions between the biology of flowering and pollination processes as well as the physical nature of pollen transport in the atmosphere. To quantify this risk, we have developed biological models of maize pollen production and viability, physical atmospheric models for pollen dispersal, and a biological model of pollen-silk interaction leading to kernel formation. We will show how these biological and physical models are linked to predict out-crossing events associated with adventitious pollen production and transport. Examples include results from field trials designed for production of non-transgenic grain, hybrid seed, and pharmaceuticals.