

BREAK-OUT GROUPS BY CROP TYPE AND CROP-OF-INTEREST

- I. Wind Pollinated Crops (e.g., corn)
- II. Self-Pollinated Crops (e.g., rice)
- III. Insect-Pollinated Crops (e.g., safflower)

BREAK-OUT SESSION TOPICS AND QUESTIONS

A. Pollen-Mediated Gene Flow Confinement

During Break-Out Session A, scientific information related to confinement of pollen-mediated gene flow from crop field tests will be discussed. First, participants will establish the basic biology of pollen for the crop-of-interest and the pollen recipient (or donor) taxonomic groups of concern in the United States, that is, other crops, weeds, or/and wild relatives. Next, the strengths and weaknesses of different confinement methods that are being used or being considered for use will be covered. Methods to be discussed include the following: physical confinement methods, (e.g., spatial isolation, border rows, wind breaks, bagging, flower removal, etc.); temporal confinement methods (e.g., planting date, harvest date, growing degree days, crop variety, etc.); and biological confinement methods (e.g., male sterility, chloroplast transformation, etc.). Participants will evaluate each method in terms of effectiveness, variability under different conditions (e.g., field test scale, weather conditions, etc.), durability (e.g., ability to withstand human error, environmental variability, etc.), and feasibility (e.g., time and cost to implement, etc.), among other characteristics. Modeling of pollen mediated gene flow, and experimental methods to measure and monitor pollen-mediated gene flow will also be deliberated on. Finally, participants will be asked to summarize their discussion by identifying emergent principles of confinement of pollen-mediated gene flow from the crop-of-interest and from crops with related mechanisms of pollination, and research needs.

- 1)** For the crop of interest, what are the pollen characteristics under typical field conditions that influence pollen mediated gene flow (e.g., pollination mechanism(s), duration of pollen viability)? Is pollen mediated gene flow influenced by variation in these characteristics under different conditions?
- 2)** Besides confinement of pollen-mediated gene flow from the field tested crop to other crop plants of the same species, does the crop hybridize with wild or weedy relatives in the U.S.?
 - a)** Do wild or weedy relatives of the crop occur in the U.S.?
 - b)** Is there compatibility in the field between the crop and relatives (i.e., compatibility of time of flowering, pollination mechanisms)?
 - c)** What is the spatial overlap of crop and relative (e.g., within field vs. external to field)? In what regions?

- d)** What other factors influence incidence of wild and weedy relatives and hybridization with the crop-of-interest (e.g., agricultural management conditions that influence hybridization or weedy relative incidence)?
- e)** Does incoming pollen flow (crop as female) pose a risk of breach of confinement for the gene being tested, or is outgoing pollen flow (crop as male) the only concern (e.g., possible detrimental consequences of hybrid seed formation on subsequent years of field testing)?
- 3)** For the crop-of-interest, what pollen-mediated gene flow confinement measures are being employed or considered? In cases in which temporal confinement is a possibility, how useful and available is information on the growing degree days or other similar indicators?
- 4)** For each of the pollen-mediated gene flow confinement measures in use or being considered, what is the effectiveness of the confinement measure under typical field conditions? How has effectiveness been determined (i.e., what data support a particular level of effectiveness)? How can pollen-mediated gene flow confinement be tested or monitored for in situ?
- 5)** How does each confinement measure vary under different conditions (e.g., distance, environmental conditions, variety being grown, scale of plot, other variables)? What are the possible mechanisms of confinement break down (e.g., human error, environmental variability, biological variability, use over multiple seasons), how likely are these, and how can break down be mitigated? If applicable, are there differences between confinement achieved with each measure with gene flow to weedy or wild relatives versus other crops?
- 6)** What is the state of the art for modeling of pollen-mediated gene flow, the effects of confinement measures, and other relevant variables (e.g., extrapolation of pollen-mediated gene flow beyond measured distances, effects of varying source and sink sizes, effects of combining different pollen confinement strategies)? Do any results from modeling impact previous responses?
- 7)** How feasible is each confinement measure and how does feasibility vary with conditions (e.g., scale)?
- 8)** What are the main ideas that emerge regarding confinement of pollen mediated gene flow from the crop-of-interest? Which steps are most critical for achieving pollen confinement? Which are most likely to break down? Does combining different methods of pollen confinement always lead to additive effects? (e.g., Does use of two methods that each account for 90% confinement, lead to a total of 99% confinement?)
- 9)** Which confinement principles are generally applicable to the crop class? What factors

do not apply generally and why?

10) What are outstanding research needs to better inform confinement measures for pollen-mediated gene flow for the crop of interest and similar crops?

B. Control of Volunteer Plants and Confinement of Seed-Mediated Gene Flow

During Break-Out Session B, scientific information related to control of volunteer plants and confinement of seed-mediated gene flow from crop field tests will be discussed. First, participants will establish the basic biology of seeds and, if applicable, vegetative propagules for the crop-of-interest. Next, the strengths and weakness of methods to control seeds and residual volunteer plants within former field test plots will be discussed. Methods to be covered include physical volunteer control methods (e.g., tillage protocols, herbicide and fire use, flooding, etc.), biological volunteer control methods (e.g., seed sterility, induced expression, etc.), and volunteer monitoring protocols. Participants will evaluate each method or protocol in terms of effectiveness, variability under different conditions (e.g., field test scale, soil conditions, etc.), durability (e.g., ability to withstand human error, environmental variability, etc.), and feasibility (e.g., time and cost to implement, etc.), among other characteristics. Subsequently, mechanisms of seed dispersal outside of the field test plots and ways to mediate it will be discussed, including unintentional co-mingling of regulated seed with other seed, equipment-mediated dispersal, and animal-mediated dispersal. As with previous topics, each confinement or control method or protocol will be evaluated in terms of effectiveness, variability, durability, and feasibility. In addition, modeling of volunteer formation and seed mediated gene flow, and experimental methods to measure and monitor seed-mediated gene flow will also be deliberated on. Finally, participants will be asked to summarize their discussion by identifying emergent principles of confinement of volunteers and seed-mediated gene flow from the crop-of-interest and from crops with related mechanisms of pollination, and research needs related to seeds and volunteers.

- 1)** What are the seed germination and dormancy characteristics for the crop of interest under typical field conditions? What factors effect germination and dormancy and in what ways?
- 2)** For the crop-of-interest, what volunteer control measures and monitoring protocols are being employed or considered?
- 3)** For each of the volunteer control measures in use or being considered, what is the effectiveness of the measure under typical field conditions? What levels of effectiveness are associated with different lengths of monitoring time for volunteers and do effectiveness levels differ geographically? What levels of effectiveness are associated with removal of volunteers at different times in the plant life cycle? How has effectiveness been determined (i.e., what data support a particular level of effectiveness)? Is planting of non-transgenic seeds in test strips in the field site an effective way to test volunteer control methods in situ? What are other possibilities for testing volunteer control methods?
- 4)** How does each control measure vary under different conditions (e.g., depth of tilling,

soil conditions, scale of plot, subsequent crops, other land use restrictions, variety being grown, other variables)? What are the possible mechanisms of volunteer confinement break down (e.g., human error, environmental variability, biological variability, use over multiple seasons), how likely are these, and how can break down be mitigated? If applicable, are there differences between volunteer control achieved with each measure if gene flow from weedy or wild relatives versus other crop individuals occurs?

5) How feasible is each volunteer control measure and how does feasibility vary with conditions (e.g., scale)?

6) What is the state of the art for modeling of volunteer emergence, the effects of confinement/control measures, and other relevant variables (e.g., modeling of weather effects on volunteer emergence)? Do any results from modeling impact on the previous responses?

7) What are points during the field testing process of seed dispersal or co-mingling (or, if relevant, vegetative propagule dispersal) related with seed handling, equipment, and transportation? How likely are these and what causes them (e.g., mechanical design, storage design, human error)?

8) What are points during the field testing process of dispersal of viable seed (or, if relevant, vegetative propagule dispersal) related with animals, weather, and other non-human factors? What animals may be involved and what are the possible volumes of seeds dispersed, dispersal distance, and likelihood of dispersal to a propagative environment?

9) What are possible mitigation measures/protocols to prevent seed dispersal or co-mingling during handling, or seed dispersal by animals or other factors and what is the effectiveness of each mitigation measure? How has effectiveness been determined (i.e., what data support a particular level of effectiveness)? How can seed confinement be tested or monitored for in situ?

10) How does each control measure for seed dispersal vary under different conditions (e.g., regional variation, other variables)? What are the possible mechanisms of confinement break down (e.g., human error, environmental variability, biological variability), how likely are these, and how can break down be mitigated?

11) How feasible is each mitigation measures/protocol for seed dispersal and how does feasibility vary with conditions (e.g., scale)?

12) What is the state of the art for modeling of seed handling methods and for modeling of seed dispersal by animals and other factors, the effects of control/confinement measures, and other relevant variables? Do any results from modeling impact previous

responses?

13) What are the main ideas that emerge regarding confinement/control of seeds and volunteer plants of the crop of interest? Which steps are most critical for achieving confinement? Which are most likely to break down? Does combining different methods of volunteer and seed confinement always lead to additive effects? (e.g., Does use of two methods that each account for 90% confinement, lead to a total of 99% confinement?)

14) Which of these ideas are generally applicable to the crop class? What factors do not apply and why?

15) What are the outstanding research needs to better inform methods of seed and volunteer confinement for the crop of interest and similar crops?

C. Overall Strategies of Confinement

During Break-Out Session C, participants will be asked to conduct an overall analysis of information and protocols related to confinement of crop field tests. Critical points of confinement during field testing and strengths and weaknesses of various confinement protocols will be identified and compared. Participants will discuss the possibility of positive or negative interactions between different confinement methods under various conditions. Redundancy of methods and other ways to mitigate weaknesses in confinement will be deliberated on. Also, experimental testing and modeling of the field test process and various confinement methods in combination will be covered. Participants will be asked to identify principles of confinement that may be generalized across different stages of a field test and among different crop types.

1) For the overall field testing process, what are the most critical points for pollen, vegetation, and seed confinement?

2) Do measures for confinement or control of different stages of field tests positively or negatively impact the effectiveness, durability, or feasibility of other methods (e.g., do any of the measures to confine pollen affect measures to control seeds?)?

3) What are the strengths and weaknesses of potential combinations of confinement measures, i.e., confinement protocols? Are there essential components that emerge for all stages (e.g., bookkeeping)?

4) How are confinement protocols impacted by various conditions (e.g, field scale, environmental conditions)?

5) What are easier points during the field testing process to build in redundancy to confinement protocols? What are critical points to build in redundancy? Which measures are most easily combined? Besides redundant measures are there other ways of mitigating weak points in confinement protocols?

- 6)** What is the state of the art for modeling of gene dispersal and confinement throughout the field testing cycle? Do any results from modeling impact previous discussion?
- 7)** What are features of a well designed experiment to test confinement strategies? How can confinement of active field test be efficiently and effectively monitored? What are the pros and cons of various monitoring methods and sampling strategies? Which steps of a field trial would benefit most from in situ measurement/verification?
- 8)** Are there principles of confinement that emerge from discussion of one class of crops (e.g., wind-pollinated) that may be general for confinement of all crops?