Problem formulation for GM crops with high potential for dispersal

Alan Raybould, Product Safety, Syngenta
Dispersal and gene flow

- Dispersal and gene flow are not the same
- Dispersal is the movement of pollen and seeds
- Gene flow is the “introduction of genetic material (by interbreeding) from one population of a species to another, thereby changing the composition of the gene pool of the receiving population.” (Encyclopaedia Britannica)
Dispersal and gene flow

- Gene flow is assumed to be the “purpose” of dispersal in plants
  - Pollen must effect fertilisation
  - Seeds must germinate and produce reproductive plants

- Dispersal = potential gene flow

- Fertilisation and production of fertile plants = actual gene flow

- For GM crop ERA, both may need to be considered, but it is important not to confuse them
Dispersal via pollen

- Plants have mechanisms to disperse pollen
- Some species use wind
  - Small, unspectacular flowers
  - Produce large amounts of pollen

Conifers

Broad-leaved trees

Grasses
Dispersal via pollen

- Some plants use animal vectors
  - Insects (birds, bats, other mammals, slugs…)
  - Attract animals by colour, scent and rewards (pollen and nectar)

Bee orchid – mimicry

Dog rose - nectar
Many crops have retained pollen dispersal mechanisms

Maize – wind dispersal

Cotton – insect dispersal (nectar)
Dispersal *via* seed

- Many mechanisms to promote seed dispersal
- Wind
  - Structures to keep seeds airborne
- Animals
  - Structures to catch the fur of animals
  - Fruits + hard seed coat to protect seeds from digestion
- Man
  - Weeds mimic crops
- Dispersal in time
  - Dormancy
Dispersal *via* seed

- **Dandelion** - wind
- **Tomato** – fruit and hard seeds
- **False oat** - mimicry
- **Goose grass** – animal fur
Many crops have lost seed dispersal mechanisms

Teosinte

- Seeds released from plant
- Seeds survive passage through gut

Maize

- Seeds retained on plant
- Seeds do not survive passage through gut
Methods to estimate dispersal and gene flow

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameter estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed traps¹</td>
<td>Potential gene flow <em>via</em> seed</td>
</tr>
<tr>
<td>Pollen traps¹</td>
<td>Potential gene flow <em>via</em> pollen</td>
</tr>
<tr>
<td>Male-sterile bait plants¹</td>
<td>Potential gene flow <em>via</em> pollen</td>
</tr>
<tr>
<td>Parentage analysis¹</td>
<td>Actual gene flow <em>via</em> pollen</td>
</tr>
<tr>
<td>Plants with genetic markers¹</td>
<td>Actual gene flow <em>via</em> seeds and pollen</td>
</tr>
<tr>
<td>Genetic structure²</td>
<td>Actual gene flow <em>via</em> seeds and pollen</td>
</tr>
</tbody>
</table>

¹Direct methods: estimate contemporary dispersal; make few assumptions; weak at detecting rare, long-distance dispersal

²Indirect method: estimates of gene flow over many generations; detect effects of rare, long-distance dispersal; rely on (usually unrealistic) assumptions about demography and genetic markers
Dispersal, gene flow and risk

- Dispersal and gene flow are natural phenomena
- Dispersal of a transgene, a transgene product or a transgenic plant is not in itself harmful
  - Although it may breach regulations

- The risk results from any hazardous properties of the transgene, the transgene product or transgenic plant
  - Hazard = potential for the transgene to cause harm
  - Exposure = presence of the gene where it may cause harm

- Dispersal and gene flow estimates are part of the exposure assessment
  - Routes by which harm may occur, not harm itself
Environmental risk and gene flow

- Define potential harmful effects of the GM crop (P₁)
- Develop hypotheses that the GM crop is not harmful (TS₁)
- Test the hypotheses (EE)
- Increased knowledge of risk (P₂)
- New risk hypothesis (TS₂)
Defining harm – an example

- Protection goals (management objectives) of laws and regulations
- USA – Plant Protection Act regulates plant pests
- United States Department of Agriculture treats GM crops as potential plant pests under the PPA
- The USDA “safeguards agriculture and natural resources from the risks associated with the entry, establishment, or spread of animal and plant pests and noxious weeds”, and thereby “ensures an abundant, high quality, and varied food supply, ... and contributes to the preservation of the global environment”
Defining harm – an example

● Clear assessment endpoints may be derived from the goal of protecting an abundant, high quality, and varied food supply
  - Abundance – crop yield
  - High-quality – nutrient content of crops (or other valued quality)
  - Varied – diversity of crops

● Reductions in these attributes could be regarded as harmful effects
Defining harm – an example

- Assessment endpoints are not so clear for preservation of the global environment
  - Abundance of non-pest species, particularly those that provide useful functions (biological control, pollination etc.) or are endangered
  - Indices of soil function

- Reductions in these attributes could be regarded as harmful effects

- (Environmental Impact Assessments might consider other things such as greenhouse gas emissions, sustainability of rural economies etc.)
Conceptual models – how could dispersal cause harm?

- Crop-to-crop gene flow by pollen
  - e.g., adverse effect on crop quality
- Crop-to-crop gene flow by seed
  - e.g., adverse effect on crop quality
  - e.g., adverse effect on crop yield
- Crop to wild relative gene flow by pollen
  - e.g., adverse effect on non-pest species
- Crop gene flow by seed to non-agricultural habitats
  - e.g., adverse effect on non-pest species
  - Crop *dispersal* by pollen
  - e.g., adverse effect on non-pest species
  - Crop *dispersal* by seed
  - e.g., adverse effect on soil function

Produce a generic conceptual model for each category.
## Crop-to-crop gene flow by pollen

<table>
<thead>
<tr>
<th>Conceptual Model</th>
<th>Risk Hypothesis</th>
</tr>
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<tbody>
<tr>
<td>GM crop produces pollen</td>
<td>GM crop does not produce pollen</td>
</tr>
<tr>
<td>Pollen disperses to neighbouring crops</td>
<td>Pollen does not disperse to neighbouring crops</td>
</tr>
<tr>
<td>Pollen fertilises neighbouring crops</td>
<td>Pollen does not fertilise neighbouring crops</td>
</tr>
<tr>
<td>Transgenic protein is produced in seed</td>
<td>Transgenic protein is not produced in seed</td>
</tr>
<tr>
<td>Transgenic protein reduces crop quality</td>
<td>Transgenic protein does not reduce crop quality</td>
</tr>
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### Hypothesis testing

1. Does the GM crop flower and produce pollen?
2. Does the pollen disperse to neighbouring crops?
3. Are neighbouring crops sexually compatible? Do they flower at the same time as the transgenic crop?
4. Developmental expression study
5. Toxicology studies/Processing studies
Crop-to-crop gene flow by seed – volunteers
## Crop-to-crop gene flow by seed – quality endpoint

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<tbody>
<tr>
<td>GM crop produces seed</td>
<td>GM crop does not produce seed</td>
</tr>
<tr>
<td>Seed disperses to neighbouring crops/ Seed disperses to subsequent crops</td>
<td>Seed does not disperse to neighbouring crops/ Seed does not disperse to subsequent crops</td>
</tr>
<tr>
<td>Seed germinates in neighbouring crops/ Seed germinates in subsequent crops</td>
<td>Seed does not germinate in neighbouring crops/ Seed does not germinate in subsequent crops</td>
</tr>
<tr>
<td>GM crop establishes</td>
<td>GM crop does not establish</td>
</tr>
<tr>
<td>GM crop produces transgenic protein</td>
<td>GM crop does not produce transgenic protein</td>
</tr>
<tr>
<td>Transgenic protein reduces crop quality</td>
<td>Transgenic protein does not reduce crop quality</td>
</tr>
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### Hypothesis testing
1. Does the crop produce seed?
2. Does the seed disperse to neighbouring/subsequent crops?
3. Germination requirements/Dormancy
4. Winter survival/Cultivation practices
5. Developmental expression study
6. Toxicology studies/Processing studies
Crop-to-crop gene flow by seed – yield endpoint

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</tr>
<tr>
<td>Seed germinates in neighbouring crops/ Seed germinates in subsequent crops</td>
<td>Seed does not germinate in neighbouring crops/ Seed does not germinate in subsequent crops</td>
</tr>
<tr>
<td>GM crop establishes</td>
<td>GM crop does not establish</td>
</tr>
<tr>
<td>GM crop affects growth of the crop</td>
<td>GM crop does not affect growth of the crop</td>
</tr>
<tr>
<td>GM crop reduces crop yield relative to effects of non-GM counterpart</td>
<td>GM crop does not reduce crop yield relative to effects of non-GM counterpart</td>
</tr>
</tbody>
</table>

Hypothesis testing
1. Does the crop produce seed?
2. Does the seed disperse to neighbouring/subsequent crops?
3. Germination requirements/Dormancy
4. Winter survival/Cultivation practices
5. Agronomic studies to compare GM with non-GM near-isolne
# Crop to wild relative gene flow by pollen

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<tr>
<td>GM crop produces pollen</td>
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<tr>
<td>Pollen disperses to wild relative</td>
<td>Pollen does not disperse to wild relative</td>
</tr>
<tr>
<td>Pollen fertilises wild relative</td>
<td>Pollen does not fertilise wild relative</td>
</tr>
<tr>
<td>Transgene is stably introgressed</td>
<td>Transgene is not stably introgressed</td>
</tr>
<tr>
<td>Wild relative produces transgenic protein</td>
<td>Wild relative does not produce transgenic protein</td>
</tr>
<tr>
<td><strong>Scenario 1</strong></td>
<td></td>
</tr>
<tr>
<td>Wild relative is the food plant of a valued non-pest species</td>
<td>Wild relative is not the food plant of a valued non-pest species</td>
</tr>
<tr>
<td>The transgenic protein is toxic to that species</td>
<td>The transgenic protein is not toxic to that species</td>
</tr>
<tr>
<td>The species receives a harmful dose of protein</td>
<td>The species does not receive a harmful dose of protein</td>
</tr>
<tr>
<td>The abundance of the species is reduced</td>
<td>The abundance of the species is not reduced</td>
</tr>
<tr>
<td><strong>Scenario 2</strong></td>
<td></td>
</tr>
<tr>
<td>The transgenic protein increases resistance to a stressor</td>
<td>The transgenic protein does not increase resistance a stressor</td>
</tr>
<tr>
<td>Increased resistance leads to higher seed production</td>
<td>Increased resistance does not lead to higher seed production</td>
</tr>
<tr>
<td>Higher seed production increases abundance of the wild relative</td>
<td>Higher seed production does not increase abundance of the wild relative</td>
</tr>
<tr>
<td>Increased abundance reduces abundance of valued species</td>
<td>Increased abundance does not reduce abundance of valued species</td>
</tr>
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Crop to wild relative gene flow by pollen

- Scenario 1
  - Gene flow as scenario 2
  - Effects testing similar to that for the crop as presented by Joerg

- Scenario 2
  - Will illustrate with example of turnip mosaic virus (TuMV) resistant oilseed rape (*Brassica napus*) in the United Kingdom
  - Shows that risk hypotheses are testable
  - Not intended to show what *should* be done for a regulatory risk assessment
TuMV in *Brassicas*

- TuMV causes yield losses in oilseeds and vegetables

- Harm if resistance transgene spreads to wild relatives and leads to increased invasiveness and loss of valued species
Species assessed

*Brassica napus* – oilseed rape

*B. oleracea* – wild cabbage

*B. nigra* – black mustard

*B. rapa* – wild turnip
### Risk hypotheses

<table>
<thead>
<tr>
<th>Conceptual model (scenario)</th>
<th>Risk hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybridisation between the crop and the wild species</td>
<td>No hybridisation between the crop and the wild species</td>
</tr>
<tr>
<td>GM trait increases the virus resistance of the wild species</td>
<td>The wild species is immune to the virus</td>
</tr>
<tr>
<td>The wild species is infected by the virus in the field</td>
<td>The virus is absent from the wild species in the field</td>
</tr>
<tr>
<td>Infected GM plants survive longer or produce more seed than infected non-GM plants</td>
<td>Infection does not reduce survival or seed production</td>
</tr>
<tr>
<td>Increased abundance of the wild species which reduces abundance of valued species</td>
<td>Population size is not limited by seed production</td>
</tr>
</tbody>
</table>
Hypothesis of no hybridisation

- Good data for all species assessed
- However *could* assess using tiered tests
- Tier I: test for hybridisation using lab methods
  - If no hybrids, stop testing; hybrids go to tier II

Hand pollination
Embryo rescue
Hypothesis of no hybridisation

- Tier II: Test for “spontaneous” hybridisation (lab or field)
  - If no hybrids, stop testing; hybrids go to tier III
Hypothesis of no hybridisation

- Tier III: Search for naturally-produced hybrids in field
  - No hybrids, stop testing
  - If hybrids, need to assess hazard
Hypothesis that wild relative is immune to TuMV
Hypothesis that wild relative is not infected in the field

Brassica nigra plants in a 400 cm² area

- TuMV
- Other viruses
- Uninfected
Hypothesis that TuMV does not reduce seed production

Number of seed per plant +/- 2SE
Hypothesis that more seed does not increase abundance

![Graph showing the effect of density on population dynamics with data points for different dates from 02/03/2000 to 20/07/2000. The graph indicates the number of plants per square meter for different densities labeled Hi, Mid, and Lo.](image)
Crop gene flow by seed to non-agricultural habitats

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<tr>
<td>Seed disperses to non-agricultural habitats</td>
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</tr>
<tr>
<td>GM crop establishes in non-agricultural habitat</td>
<td>GM crop does not establish in non-agricultural habitat</td>
</tr>
<tr>
<td>GM crop forms self-sustaining population</td>
<td>GM crop does not form self-sustaining population</td>
</tr>
<tr>
<td>Population increases in abundance</td>
<td>Population does not increase in abundance</td>
</tr>
<tr>
<td>Increased abundance harms a valued species</td>
<td>Increased abundance does not harm a valued species</td>
</tr>
</tbody>
</table>

Hypothesis testing

1. Does the crop produce seed?
2. Does the seed disperse to non-agricultural habitats?
3. Germination requirements/Dormancy
4. Winter survival/Competition with wild plants
5. Agronomic studies to compare GM with non-GM near-isoline
   ➢ (Simulate conditions in non-agricultural habitats/ conditions under which transgene may be advantageous)
## Crop dispersal by pollen

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<tr>
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</tr>
<tr>
<td>Pollen disperses outside the field</td>
<td>Pollen does not disperse outside the field</td>
</tr>
<tr>
<td>Pollen is eaten by a valued species</td>
<td>Pollen is not eaten by a valued species</td>
</tr>
<tr>
<td>Transgenic protein is toxic to the valued species</td>
<td>Transgenic protein is not toxic to the valued species</td>
</tr>
<tr>
<td>The species receives a harmful dose of protein</td>
<td>The species does not receive a harmful dose of protein</td>
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<td>The abundance of the valued species is reduced</td>
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### Impact of Bt corn pollen on monarch butterfly populations: A risk assessment

Mark K. Sears*, Richard L. Hall*†, Dana E. Sundberg*‡, Karen S. Charbonneau, John M. Pierson*, Heather R. Morland*, Dian D. Siegfried, and Galen P. Dively*

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†Department of Botany, University of Maryland, College Park, MD 20742, USA
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A collaborative research effort by scientists in several states and in Canada has produced information to develop a formal risk assessment of the impact of Bt corn pollen on monarch butterfly (Danaus plexippus) populations. Information was gathered from studies on which GM corn produces Bt pollen and the degree to which monarch larvae would be exposed to toxic amounts of Bt pollen in its field plant, the common nettle (Rumex obtusifolius), and another nettle species, nettle (Rumex crispus), found in and around habitat. Because of the protein's toxic effects, Bt pollen may be an important contributor to declines in monarch populations across different areas. Some studies indicate that Bt pollen is also toxic to monarchs that feed onBt corn. In the field, Bt pollen may contaminate the nectar from flowers that monarchs feed on, and this pollen may be ingested by monarchs. The potential for exposure by monarch larvae to pollen in the field is relatively low, thereby reducing any risk assessment.

In this paper, we develop a strategy to evaluate the risk of exposure of monarch larvae to Bt corn pollen and the impact of such exposure on populations of monarch butterflies in eastern North America based on research published elsewhere.
Crop dispersal by seed

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<tr>
<td>GM crop produces seed</td>
<td>GM crop does not produce seed</td>
</tr>
<tr>
<td>Seed contains a transgenic enzyme</td>
<td>Seed does not contain a transgenic enzyme</td>
</tr>
<tr>
<td>Enzyme belongs to a class known to affect soil function</td>
<td>Enzyme does not belong to a class known to affect soil function</td>
</tr>
<tr>
<td>Seed is spilled during harvest</td>
<td>Seed is not spilled during harvest</td>
</tr>
<tr>
<td>Enzyme concentration raised significantly above background</td>
<td>Enzyme concentration not raised significantly above background</td>
</tr>
<tr>
<td>Raised enzyme concentration changes rate of soil process</td>
<td>Raised enzyme concentration does not change rate of soil process</td>
</tr>
<tr>
<td>Change in rate causes harm to soil function</td>
<td>Change in rate does not cause harm to soil function</td>
</tr>
</tbody>
</table>

Hypothesis testing

- Maize producing large amounts of an enzyme involved in plant degradation
- Seed spillage likely to give instantaneous increase of 10X background
- Enzyme is induced when plant material added to soil
- Enzyme is not rate-limiting for degradation
- No change in rate of degradation expected – no harm to soil function
Conclusions

- Assessment of risks from GM crops via dispersal, gene flow, weediness and invasiveness need not be complicated
- Clearly define the harmful effects
- Create a conceptual model comprising a series of steps that must all occur for harm to be realised
Conclusions

- Formulate simple, testable hypotheses about one or more stages
  - The phenomenon does not occur
  - The phenomenon occurs at a frequency below that required for harm
  - The phenomenon occurs at a magnitude below that required for harm
  - No more than/no less than the current non-GM crop is often a useful hypothesis

- Precise predictions of transgene frequencies, numbers of transgenic hybrids, or population growth rates of transgenic weeds are unnecessary
Conclusions

- Remember to place risks in context
- Arable crops and their hybrids are rarely serious weeds or invaders
- Higher weediness or invasiveness potential in controlled studies is unlikely to result in serious harm in the field
  - Would not trigger further evaluation in weed risk assessment models