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ORIGINAL PAPER

Assessing the ecological risks from the persistence and spread of feral populations of insect-resistant transgenic maize

Alan Raybould · Laura S. Higgins · Michael J. Horak · Raymond J. Layton · Nicholas P. Storer · Juan Manuel De La Fuente · Rod A. Herman

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Abstract One source of potential harm from the cultivation of transgenic crops is their dispersal, persistence and spread in non-agricultural land. Ecological damage may result from such spread if the abundance of valued species is reduced. The ability of a plant to spread in non-agricultural habitats is called its invasiveness potential. The risks posed by the invasiveness potential of transgenic crops are assessed by comparing in agronomic field trials the phenotypes of the crops with the phenotypes of genetically similar non-transgenic crops known to have low invasiveness potential. If the transgenic and non-transgenic crops are similar in traits believed to control invasiveness potential, it may be concluded that the transgenic crop has low invasiveness potential and poses negligible ecological risk via persistence and spread in non-agricultural habitats. If the phenotype of the transgenic crop is outside the range of the non-transgenic comparators for the traits controlling invasiveness potential, or if the comparative approach is regarded as inadequate for reasons of risk perception or risk communication, experiments that simulate the dispersal of the crop into non-agricultural habitats may be necessary. We describe such an experiment for several commercial insect-resistant transgenic maize events in conditions similar to those found in maize-growing regions of Mexico. As expected from comparative risk assessments, the transgenic maize was found to behave similarly to non-transgenic maize and to be non-invasive. The value of this experiment in assessing and communicating the negligible ecological risk posed by the low invasiveness potential of insect-resistant transgenic maize in Mexico is discussed.

Keywords Ecological risk assessment · Insect resistance · Feral maize · Invasiveness potential · Field study

Introduction

Ecological risk assessments contribute crucial knowledge to regulators making decisions about whether to permit the commercial cultivation of transgenic crops. Ecological risk assessments for any proposed action begin with problem formulation (Wolt et al. 2010). Problem formulation is conceptually straightforward,

A. Raybould (✉)
Syngenta, Jealott's Hill International Research Centre,
Bracknell, Berkshire RG42 6EY, UK
e-mail: alan.raybould@syngenta.com

L. S. Higgins · R. J. Layton
Pioneer Hi-Bred International, a DuPont Business,
7250 NW 62nd Ave., Johnston, IA 50131, USA

M. J. Horak · J. M. De La Fuente
Monsanto Company, 800 N. Lindbergh Blvd.,
St. Louis, MO 63167, USA

N. P. Storer · R. A. Herman
Dow AgroSciences LLC, 9330 Zionsville Rd.,
Indianapolis, IN 46268, USA

but often difficult in practice: agree definitions of ecological harm; develop plausible scenarios about how the proposed action may result in ecological harm; and estimate the likelihood of those scenarios being realized.

One source of potential harm from the cultivation of transgenic crops is the persistence and spread of those crops as weeds of other crops or of non-agricultural land (Keeler 1989; Raybould and Gray 1993). The intrinsic ability of a crop to become a weed is called its weediness potential. Crops that persist in fields following harvest, through seed or vegetative tissue, and which appear in following crops are called volunteers. Volunteers may cause economic harm by reducing the yield or the quality of the crops they infest, and through the costs of control (Froud-Williams et al. 1993).

Some crops readily disperse outside cultivated land, often through spillage of seed during transport after harvest (Crawley and Brown 1995). Crops that establish and persist outside agriculture are described as feral, meaning the crops have reverted from a domesticated to a wild state (Gressel 2005). Feral crops may cause ecological harm if they spread and thereby reduce the abundance of valued species (Pimentel et al. 2001; Raybould and Cooper 2005). The likelihood that a crop will persist and spread in non-agricultural habitats is called its invasiveness potential.

At its simplest and most conservative, problem formulation may be thought of as the creation of hypotheses that the proposed action will not result in any ecological harm, along with the identification of sufficient tests of those hypotheses, such that if the hypotheses are corroborated by the tests, negligible risk may be concluded with confidence. This paper describes the formulation and testing of hypotheses about the probability of ecological harm from transgenic crops becoming invasive. It discusses the common practice of assessing these risks using data from agronomic field trials that compare the phenotypes of a transgenic crop and a genetically similar non-transgenic crop that is known to pose minimal ecological risk. The paper also presents a method for assessing the ecological risks from an invasive transgenic crop should the field trial data indicate that the transgenic crop has greater invasiveness potential than the non-transgenic comparator, or should the agronomic field trial data be deemed insufficient for any reason even though no increase in the weediness or invasiveness potential of the transgenic crop is indicated. The method is illustrated by a study of the invasiveness potential of several transgenic insect-resistant maize varieties.

Invasiveness potential of transgenic crops: general concepts and current regulatory practice

A generic scenario by which harm may arise from the invasion of a transgenic crop that reproduces by seed has been suggested by Raybould (2011): the transgenic crop produces seed → seeds disperse to non-agricultural habitats → the crop establishes in the non-agricultural habitat → the crop forms a self-sustaining population → the population increases in abundance → increased abundance of the crop reduces the abundance of a valued species (ecological harm). Each step in the scenario gives a testable hypothesis, which if rigorously tested and corroborated would indicate negligible ecological risk via invasion of non-agricultural land by the crop: the crop does not produce seed (perhaps because it is harvested before flowering); the crop does not disperse to non-agricultural habitats; the crop does not establish in non-agricultural habitats; and so on.

In regulatory risk assessments for the cultivation of transgenic crops, none of these hypotheses is tested directly; instead, the transgenic crop is compared with genetically similar non-transgenic crops that do not cause ecological harm through invasion of non-agricultural habitats in the region of intended cultivation of the transgenic crop. The factors preventing the non-transgenic crops from causing ecological harm may not be known precisely; however, their long history of cultivation without ecological harm has, in effect, tested and corroborated the hypotheses that the chain of events from cultivation to ecological harm is broken at one or more links. If the transgenic crop does not differ significantly from the non-transgenic crop in characters believed to determine its invasiveness potential, it may be concluded that the links are similarly broken for the transgenic crop, and, therefore, that its cultivation is unlikely to result in ecological harm via invasion of non-agricultural habitats.

The phenotypes of the transgenic crop and one or more non-transgenic comparators (also called reference materials) are compared in agronomic field trials, usually at several sites throughout the intended range of cultivation of the transgenic crop. The characters that are compared fall into 3 broad categories: growth habit and vegetative vigour; phenology and reproductive behaviour; and susceptibility to pests, diseases and abiotic stress (Horak et al. 2007; Raybould et al. 2010). Should any of the characters be statistically significantly different between the transgenic and non-transgenic crops, the differences are evaluated for whether they indicate an increase in invasiveness potential. The first stage in such an evaluation is to test whether the value of character in the transgenic crop that is statistically significantly different falls outside the range of the crop generally (Horak et al. 2007). If the value of the character in the transgenic crop falls outside the range of the crop generally, further evaluation of the putative increase in invasiveness potential, perhaps involving experiments to test directly the hypotheses indicated above, would be required.

To date, commercially available transgenic crops have been determined to pose negligible ecological risk via invasion of non-agricultural habitats based on the absence of phenotypic differences with non-transgenic comparators that would indicate an increase in invasiveness potential (e.g., McHughen and Holm 1995; Mendelsohn et al. 2003; Heck et al. 2005; Horak et al. 2007; Raybould et al. 2010). The main value of these field trials in ecological risk assessment is to test the hypothesis that invasiveness potential of the transgenic crop has not increased owing to unintended effects of transformation. Knowledge of critical factors limiting the spread of crop may be useful for assessment of whether the intended effect of transformation is likely to increase weediness potential: for example, if feral populations of the crop do not require control with herbicides, acquisition of herbicide tolerance is unlikely to increase the invasiveness potential of the crop; similarly, if insect damage is not an important factor limiting the establishment and spread of feral populations of a crop, enhanced insect resistance is unlikely to increase the invasiveness potential of the crop.

Direct tests of invasiveness potential

There may be situations when the studies described above do not give risk managers (regulators, for example) sufficient confidence of negligible ecological risk via invasion by a particular transgenic crop, or the studies may be regarded as insufficient for risk managers to communicate the risk effectively. Reasons could include the transgenic crop having a phenotype outside the range of the crop for a trait believed to influence its invasiveness potential, concern that agronomic field trials are not sufficiently predictive of the behaviour of the crop in non-agricultural habitats, or because the potential consequences of invasiveness are regarded as being especially serious, perhaps involving loss of genetic resources in crop relatives in a centre of origin or diversity. In these circumstances, one could perform a field trial that simulates the spread of the transgenic crop into the habitats of concern (Crawley et al. 1993). This paper reports such a trial.

Several insect-resistant maize events producing single insecticidal traits have been grown commercially in the United States for several years; these include Bt11 and MON810, which produce Cry1Ab to control European corn borer; TC1507, which produces Cry1F, also for control of European corn borer; and DAS59122, which produces a binary toxin comprising Cry34Ab1 and Cry35Ab1 for control of corn rootworm. During regulatory field trials, maize containing these events showed no phenotypic differences from conventional maize that indicated increased invasiveness potential (e.g., Mendelsohn et al. 2003), and there are no reports of commercially cultivated insect-resistant crops being more invasive of natural habitats than are conventional crops (e.g., Sanvido et al. 2007).

Mexico is a centre of origin and centre of diversity of maize (Hancock 1992), and hence the potential for increased weediness and invasiveness potential of transgenic maize is of particular interest there. Despite many tests in agronomic field trials that corroborated the hypothesis that transgenic insect-resistant maize is not invasive of natural habitats, and the absence of invasive transgenic insect-resistant maize in all areas where it has been cultivated, further testing of the hypothesis was requested for ecological risk assessments for cultivation of these crops in Mexico. The tests simulated the dispersal of maize into uncultivated land, and assessed its subsequent ability to form persistent, self-sustaining populations. The ability of transgenic insect-resistant maize to establish and persist was compared with that of near-isogenic non-transgenic maize and Mexican landraces to test the hypothesis that transgenic insect resistance does not increase the invasiveness potential of maize. At the time of this study, Mexico did not allow field trials of transgenic maize; therefore, the experiment was conducted in the United States under environmental conditions representative of those where maize is grown in Mexico.

Invasiveness potential study: materials and methods

The experiment was conducted between January 2006 and March 2008 at a field site near Raymondville (Willacy County), Texas USA (Fig. 1). Raymondville is in the warm Temperate Zone of the Northern Hemisphere, specifically in a Modified Marine (Subtropical Subhumid) climate (Larkin and Bomar 1983), and much of Mexico has a similarly temperate climate (Miranda and Sharp 1950). In this area, 20-year averages for rainfall and minimum and maximum temperatures are approximately 5.2 cm, 17.8 and 29.6°C, respectively. The soil is Rio sandy clay loam (Turner 1982) and the site cropping history included grain sorghum and cotton cultivation in 2005.

Fig. 1 Representative photographs of test plots. These plots are of Entry 1 (Pioneer maize hybrid 31G66, a non-genetically modified hybrid). Above, 12 days after planting (April 3, 2006); below, crop maturity (July 6, 2006)

The experiment was conducted in a randomized complete block design with 13 entries (maize hybrids or maize landraces), with three replications per entry. The 13 maize entries were five transgenic maize hybrids containing insect-resistance traits, four near-isogenic control maize hybrids (paired with the transgenic maize hybrids), three Mexican landraces as maize best adapted to the area (CIMMYT 1998; Beck 2000), and a non-transgenic locally adapted maize hybrid (see Table 1 for details).

The maize was sown at a target density of ~28,000 plants per acre (~69,000 plants per ha) in plots of approximately 110 m^2 (14.2 m by 7.7 m) with 97 cm row spacing. An unplanted buffer strip of between 5.3 and 5.5 m width was established around each plot and was included in the plot monitoring. This buffer area was unmanaged and quickly reverted to natural vegetation.

Plots were sown on March 22, 2006, and concurrently a granular insecticide (Force® 3G, tefluthrin 3.0%) was applied at 0.13 kg ai/ha to control soil pests. Typical local agronomic practices were employed to ensure the successful establishment of maize populations in the first year of the experiment. Post-emergence herbicide applications included Prowl® 3.3EC (pendimethalin 37.4%) at 0.9 kg ai/ha and atrazine 42.2% at a 1.1 kg ai/ha, both applied on April 21, 2006. No herbicides containing glyphosate or glufosinate were applied to any plot. Nitrogen applications were made on April 4 and April 20, 2006 (at 79.6 and 119.9 kg/ha, respectively). All plots were treated with Tracer® 4F (spinosad) at 0.1 kg ai/ha on May 19, 22, 26, 29 and June 5, 2006 for control of corn earworm and fall armyworm to ensure production of healthy and robust maize ears. Plots were irrigated using furrow irrigation on January 18, March 1, April 21, May 2, May 26, and June 12, 2006.

Twelve days after sowing, the number of emerged plants in each plot was recorded. The initial crop reached physiological maturity in July 2006. At maturity, the number of plants with ears in each plot was recorded. No plants were harvested or disturbed in the plots, and maize seed was allowed to disperse naturally. Each plot was evaluated for “feral maize”—

Table 1 Transgenic maize events, their near-isogenic lines and maize landraces used in the invasiveness study

Entry	Maize line	Transgene(s)	Trait
1	31G66	–	Locally adapted non-transgenic hybrid
2	NK603 × MON810	*cp4 epsps*, *cry1Ab*	Glyphosate tolerance, corn borer resistance
3	MON810	*cry1Ab*	Corn borer resistance
4	MON810 isoline	–	Non-transgenic hybrid
5	DAS 59122	*pat, cry34Ab1/cry35Ab1*	Glufosinate tolerance, corn rootworm resistance
6	DAS 59122 isoline	–	Non-transgenic hybrid
7	TC1507	*pat, cry1F*	Glufosinate tolerance, corn borer resistance
8	TC1507 isoline	–	Non-transgenic hybrid
9	Bt11	*pat, cry1Ab*	Glufosinate tolerance, corn borer resistance
10	Bt11 isoline	–	Non-transgenic hybrid
11	Landrace POP 21	–	Non-transgenic Mexican landrace 1
12	Landrace POP 502	–	Non-transgenic Mexican landrace 2
13	Landrace POP 902	–	Non-transgenic Mexican landrace 3

maize plants originating from the initial crop establishing in the now uncultivated plots and buffer strips—every 3 months for 21 months after initial crop maturity. Plot monitoring included visual inspections within the plots as well as inspection of the buffer areas within 5 m of the borders of each plot. The final plot observations were conducted in March 2008.

The replacement capacity (RC) of the maize was calculated:

$$\mathrm{RC} = \frac{\#\text{ of plants present at specific observation time}}{\#\text{ of plants present after initial sowing}}$$

A population with an RC value less than one is in decline, while a population with an RC value greater than one is increasing. RC values of the transgenic maize were compared with the RC values for the non-transgenic maize, near-isogenic control lines, and the Mexican landraces.

Invasiveness potential study: results

Weather summary

Annual average minimum and maximum air temperatures during the study were within 1.2°C of the 20-year averages. The largest deviation from the 20-year average was in January 2007, which was 4.9°C below average. Average temperatures during the study represented normal temperatures for the region. For 2006, average monthly rainfall was below the 20-year average, although for the first half of the year, the plots were supplemented with irrigation to produce a healthy crop. The second half of 2006 (July through December) was slightly above average in rainfall (110%). Rainfall in 2007 was above average for the year: January and February were significantly below average (approximately 11% of the 20-year average), but May, July, and September were particularly wet, bringing the average rainfall for the year to over 130% of the 20-year historical average. Apart from summer 2007, rainfall during the study was representative of the area, and during the study the weather was conducive to the establishment of maize.

Crop establishment, growth and reproduction

Evaluation of early stand counts for each entry in the study at 10–14 days after planting, as well as at crop maturity, indicated that all hybrids and landraces were established as productive stands that grew to maturity (Table 2). All entries had a high percentage (83–97%) of early stand plants that grew to maturity, that is, they produced at least one ear. Ears produced on these plants had abundant kernels from which subsequent generations could arise (Fig. 2).

Maize population dynamics and replacement capacity

Feral maize population dynamics were similar across all entries in the study (Table 2). After the initial crop

Table 2 Feral maize population dynamics

Maize line	Average number of plants per plot								
	10–14 DAP	Maturity[a]	3 MAM	6 MAM	9 MAM	12 MAM	15 MAM	18 MAM	21 MAM
31G66	651	597	0	23	2	0	0	0	0
NK603 × MON810	619	567	0	14	8	0	0	0	0
MON810	642	625	0	46	3	0	0	0	0
MON810 isoline	647	614	6	30	4	0	0	0	0
DAS 59122	654	617	0	7	1	0	0	0	0
DAS 59122 isoline	634	553	0	1	0	0	0	0	0
TC1507	646	628	0	3	0	0	0	0	0
TC1507 isoline	656	634	1	14	1	0	0	0	0
Bt11	648	598	0	8	0	0	0	0	0
Bt11 isoline	655	602	1	17	0	0	0	0	0
Landrace POP 21	621	580	1	7	2	0	0	0	0
Landrace POP 502	646	614	8	95	7	0	0	0	0
Landrace POP 902	655	541	9	52	4	0	0	0	0

DAP days after planting, *MAM* months after maturity

[a] Plants with at least one fully developed ear

matured, the plants dried, and stalks and ears within husks fell into the plot area. No measures were taken to remove the husks, remove kernels from the ear, or disperse the kernels as these processes would be left to natural means in a feral population, should it become established.

Feral maize plants emerged from seed from the fallen ears. For all entries, feral plants were observed during the first 12 months after initial crop maturation, with maximum numbers reached after 6 months. At all observation times, for all entries, the average population per plot was much less than the initial plant population (fewer than 100 feral maize plants per plot versus initial plant populations between 610 and 660 plants per plot; Table 2). Two of the three Mexican landraces which are adapted to the area had the highest number of feral plants at 6 months and averaged 95 plants per plot (landrace 2) and 52 plants per plot (landrace 3) (Table 2). Assessment of feral plant populations of the transgenic maize hybrids in relation to their control hybrids showed no trend for higher transgenic plant survival: all populations were of fewer than 50 plants per plot, and in 3 of 5 cases the control hybrids had larger populations than the transgenic hybrids. After 6 months, the number of feral maize plants declined rapidly, and by 12 months post maturity, feral maize was absent from the plots and was not detected for the remainder of the study. Two years after the initiation of the experiment, the plot areas had reverted to native vegetation (Fig. 3).

Fig. 2 Representative maize ears from test plots. The photographed ears were husked back to show kernel development

Few feral maize plants reached reproductive maturity, and those that did were observed 6 months after initial crop maturation. At the 6-month observation, the hybrid entries (whether transgenic or control) had fewer than 1 plant per plot on average that reached reproductive maturity while the landraces had fewer than 2 plants per plot on average that reached reproductive maturity.

RC values were calculated for feral maize for four periods during the study. At all observation times the

Fig. 3 Photograph of the experimental site approximately 24 months after initial planting (the end of study). The site has reverted to native vegetation. One of the replicate plots of the Mexican landrace POP 502 is shown

RC values were well below 1, indicating populations in decline (Table 3). For all entries and across observation times from 12 months after sowing through the termination of the study (21 months after sowing), the RC was zero, indicating no viable plants were observed in the field and the populations had died out. There were no differences in the RC values between the transgenic maize entries and the non-transgenic control lines. This indicated that the transgenic traits were not imparting an advantage to maize that would allow it to survive better than the control in unmanaged environments.

Discussion

Ecological risks from invasive transgenic insect-resistant maize

Modern maize (*Zea mays* L.) is highly domesticated, originating from human selection of teosinte more than 8,000 years ago (Galinat 1988). The extensive modification of maize from teosinte has rendered it unable to establish self-sustaining populations outside agriculture (Wallace and Brown 1988; Hoeft et al. 2000; OECD 2003). Maize does not establish self-sustaining feral populations for several reasons: it has poor seed dispersal because its seeds do not dehisce from the cob; it lacks seed dormancy, and therefore tends to germinate in the autumn, resulting in failure to overwinter in cold climates (Palaudelmàs et al. 2009); and it is a poor competitor with native perennial vegetation, which outcompetes it for light, nutrients and water (Olson and Sander 1988).

Because of its inability to form self-sustaining feral populations, cultivation of maize poses negligible ecological risk to uncultivated areas. Ecological risk assessments for cultivation of transgenic maize test the hypothesis that the event in question has not changed the crop phenotype in traits thought to control its invasiveness potential, through either intended or unintended effects of transformation. If this hypothesis is corroborated, the transgenic maize is no more likely than conventional maize to invade non-agricultural habitats, and may be deemed to pose negligible

Table 3 Replacement capacity (RC) of feral maize calculated for each observation period after initial crop maturity

Maize line	3 months	6 months	9 months	12–21 months[a]
31G66	0.00	0.04	0.00	0.00
NK603 × MON810	0.00	0.03	0.01	0.00
MON810	0.00	0.07	0.00	0.00
MON810 isoline	0.01	0.05	0.01	0.00
DAS 59122	0.00	0.01	0.00	0.00
DAS 59122 isoline	0.00	0.00	0.00	0.00
TC1507	0.00	0.00	0.00	0.00
TC1507 isoline	0.00	0.02	0.00	0.00
Bt11	0.00	0.01	0.00	0.00
Bt11 isoline	0.00	0.03	0.00	0.00
Landrace POP 21	0.00	0.01	0.00	0.00
Landrace POP 502	0.01	0.16	0.01	0.00
Landrace POP 902	0.02	0.10	0.01	0.00

[a] Includes observations 12, 15, 18, and 21 months after initial crop maturity

ecological risk outside cultivation via this mechanism. Corroboration would also further suggest that ecological and population genetic risks posed by hybridisation between transgenic insect-resistant maize and wild relatives are negligible (Hokanson et al. 2010).

For regulatory risk assessments, the hypothesis of no increased weediness potential in transgenic maize is tested routinely in agronomic field trials that allow comparison of the vegetative vigour, phenology, reproductive behaviour, and susceptibility to pests, diseases and abiotic stress of the transgenic maize with one or more suitable non-transgenic comparators (Horak et al. 2007); dormancy and germination may also be compared in laboratory studies (e.g., Raybould et al. 2010). Similarity in these characters between the transgenic and non-transgenic maize corroborates the hypothesis of no increased weediness potential, and thereby indicates that the transgenic maize will pose negligible ecological risk in non-agricultural habitats owing to its inability to spread to and establish in those areas.

The comparative approach to the assessment of ecological risks of feral transgenic maize is usually sufficient for regulatory decision-making; direct measurement of the invasiveness of transgenic maize is not usually required. The experiment reported here was a direct test of the ability of several transgenic insect-resistant maize events to form feral populations under Mexican environmental conditions. The study was not triggered by findings of potentially harmful differences in comparative studies, but by a request for additional testing of the hypothesis of negligible risk from invasive feral transgenic maize owing to high concern about potential adverse effects on maize genetic diversity in its centre of origin.

The results of the study corroborate the hypothesis that transgenic insect-resistance traits do not increase the invasiveness potential of maize relative to non-transgenic maize, either through the intended effects of the traits or through harmful unintended effects of transformation. As expected from comparisons of characters associated with invasiveness potential conducted in agronomic field trials and in laboratory studies, the presence of insect-resistance traits did not increase the RC values of the transgenic maize hybrids. We conclude that in the environment of south Texas and in similar environments in Mexico, transgenic insect-resistant maize plants would be no better at establishing populations in an unmanaged environment than would non-transgenic maize; thus, cultivation of these events would pose negligible risk to the environment.

Judging the sufficiency of data for risk assessment

Regulatory risk assessments test hypotheses that a proposed action will not lead to harmful effects that are specified in, or deduced from, laws, policies or regulations. For risks from cultivating transgenic crops, the hypotheses are of two kinds: the intended phenotypic change to the crop will have no harmful side-effects; and there are no potentially harmful unintended changes resulting from transformation. Such risk hypotheses can never be proved because it is always possible that a harmful effect will result despite extensive testing and corroboration of the hypotheses. It follows that the amount of testing of risk hypotheses required for regulatory decision-making is a matter of judgement, not of scientific analysis (Raybould 2011). Regulators must balance the costs of too much testing of activities that pose low risk with those of too little testing of activities that appear to pose low risk, but which further testing would have shown to pose high risk (Caley et al. 2006; Chapman et al. 1998).

Many of the tests carried out for regulatory risk assessments for the cultivation of transgenic crops are conducted because it was thought that transgenesis might produce harmful unintended effects more often than would other methods of plant breeding, such as hybridization and mutagenesis, used to introduce phenotypic variation. Numerous molecular studies [reviewed by Ricroch et al. (2011)], and many years' experience of selecting and breeding transgenic crops (Bradford et al. 2005), have extensively tested and corroborated the hypothesis that harmful unintended effects are no more likely to arise from transgenesis than from other methods of plant breeding. It is argued, therefore, that molecular analyses that test for potentially harmful unintended effects of transgenesis should no longer be required routinely (Herman et al. 2011). Similarly, compositional analysis may not be required for transgenic crops with input traits (e.g., herbicide tolerance and insect resistance) conferred by the production of a single protein with a discrete mode of action, although compositional analysis may still be required for crops with output traits where the genetic modification is intended to change biochemical pathways (Herman et al. 2009). The argument is also relevant for invasiveness potential: transgenic crops *per se* are no more likely to become invasive than are non-transgenic crops with similar phenotypes introduced by other methods, and studies of the invasiveness potential of a transgenic crop should not be required unless there is reason to believe that the introduced traits will increase the invasiveness potential of the crop.

Hypotheses that the intended trait will not increase invasiveness potential of a crop can often be tested adequately without field studies that simulate dispersal of transgenic crop seed outside agriculture. If the crop does not form self-sustaining feral populations, knowledge of the factors that prevent establishment and persistence of the crop may be a sufficient test. Maize is unable to establish outside agriculture because of poor dispersal, lack of dormancy and competition from perennial plants (see above), not because insects prevent feral maize plants from establishing or reproducing; therefore, accumulated observations on maize dispersal and competitive ability could be considered a sufficient test of the hypothesis that transgenic insect-resistant maize will not be invasive and, if so, a field study such as described here would not be required.

Finally, although existing data may be used to test risk hypotheses and indicate negligible probabilities of harmful effects through invasiveness of transgenic crops, certain new studies may be conducted because they make risk communication easier or change the perception of risk more effectively than arguments based on existing knowledge. Sjöberg (2004) makes two relevant points: first, “interference with nature” is an important element in the perceived risks of transgenic crops; and secondly, the probabilities of harmful events (i.e., risks) may be “hard to understand, and are based on elaborate and debatable models and assumptions”. If transgenic crops are seen as interference with nature, and if comparative risk assessment using data from field trials seems elaborate and debatable, a single experiment that *shows* a transgenic crop being overwhelmed by natural vegetation may be attractive to risk managers and decision-makers, regardless of whether it is triggered by significant uncertainty about risk.

In summary, an experiment that simulated the dispersal of maize seed into non-agricultural land under environmental conditions found in parts of Mexico showed, as expected, that maize was unable to form self-sustaining feral populations, and that transgenic insect-resistance did not increase the invasiveness potential of maize. The experiment increases confidence that the invasiveness potential of transgenic maize is predictable from agronomic field trials that compare the phenotypes of transgenic and non-transgenic maize. While the results of the experiment were unsurprising, the experiment may be useful for communicating the negligible ecological risks from invasive transgenic insect-resistant maize in Mexico.

References

Beck D (2000) Research on tropical highland maize. Maize Research Highlights 1999–2000. CIMMYT. México, DF, México. Available at http://apps.cimmyt.org/Research/Maize/results/mzhigh99-00/mrhigh99-00_res.pdf

Bradford KJ, Van Deynze A, Gutterson N, Parrott W, Strauss SH (2005) Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. Nat Biotechnol 23:439–444

Caley P, Lonsdale WM, Pheloung PC (2006) Quantifying uncertainty in predictions of invasiveness, with emphasis on weed risk assessment. Biol Invasions 8:1595–1604

Chapman PM, Fairbrother A, Brown D (1998) A critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environ Toxicol Chem 17:99–108

CIMMYT (1998) A complete listing of improved maize germplasm from CIMMYT. Maize Program Special Report. CIMMYT. México, DF, México. Available at http://libcatalog.cimmyt.org/download/cim/67094.pdf

Crawley MJ, Brown SL (1995) Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. Proc R Soc Lond B Biol Sci 271:1909–1916

Crawley MJ, Hails RS, Rees M, Kohn D, Buxton J (1993) Ecology of transgenic oilseed rape in natural habitats. Nature 363:620–623

Froud-Williams RJ, Knott CM, Lutman PJW (eds) (1993) Volunteer crops as weeds. Association of Applied Biologists, Warwick

Galinat WC (1988) The origin of corn. In: Sprague GF, Dudley JW (eds) Corn and corn improvement, 3rd edn. American Society of Agronomy, Madison, pp 1–31

Gressel J (ed) (2005) Crop ferality and volunteerism. CRC Press, Boca Raton

Hancock JF (1992) Plant evolution and the origin of crop species. Prentice Hall, Englewood Cliffs

Heck GR, Armstrong CL, Astwood JD, Behr CF, Bookout JT, Brown SM, Cavato TA, DeBoer DL, Deng MY, George C, Hillyard JR, Hironaka CM, Howe AR, Jakse EH, Ledesma

BE, Lee TC, Lirette RP, Mangano ML, Mutz JN, Qi Y, Rodriguez RE, Sidhu SR, Silvanovich A, Stoecker MA, Yingling RA, You J (2005) Development and characterization of a CP4 EPSPS-based glyphosate-tolerant corn event. Crop Sci 44:329–339

Herman RA, Chassy BM, Parrott W (2009) Compositional analysis of transgenic crops: an idea whose time has passed. Trends Biotechnol 27:555–557

Herman RA, Song P, Zhuang M (2011) Safety risks of cryptic reading frames and gene disruption due to crop transgenesis. GM Crops 2:4–6

Hoeft RG, Nafziger ED, Johnson RR, Aldrich SR (2000) Modern corn and soybean production. MCSP Publications, Champaign

Hokanson KE, Ellstrand NC, Ouedraogo JT, Olweny PA, Schaal BA, Raybould AF (2010) Biofortified sorghum in Africa: using problem formulation to inform risk assessment. Nat Biotechnol 28:900–903

Horak MJ, Rosenbaum EW, Woodrum CL, Martens AB, Mery RF, Cothren JT, Burns JA, Nickson TE, Pester TA, Jiang C, Hart JL, Sammons B (2007) Characterization of Roundup Ready Flex cotton, ‘MON88913’, for use in ecological risk assessment: evaluation of seed germination, vegetative and reproductive growth, and ecological interactions. Crop Sci 47:268–277

Keeler KH (1989) Can genetically engineered crops become weeds? Bio/technology 7:1134–1137

Larkin TJ, Bomar GW (1983) Climatic atlas of Texas. Texas Department of Water Resources, Austin

McHughen A, Holm FA (1995) Transgenic flax with environmentally and agronomically sustainable attributes. Transgenic Res 4:3–11

Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2003) Are *Bt* crops safe? Nat Biotechnol 21:1003–1009

Miranda F, Sharp AJ (1950) Characteristics of the vegetation in certain temperate regions of eastern Mexico. Ecology 31:313–333

OECD (Organisation for Economic Cooperation and Development) (2003) Consensus document on the biology of *Zea mays* subsp. mays. Series on Harmonisation of Regulatory Oversight in Biotechnology, No 27. Available at http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono(2003)11&doclanguage=en

Olson RA, Sander DH (1988) Corn production. In: Sprague GF, Dudley JW (eds) Corn and corn improvement, 3rd edn. American Society of Agronomy, Madison, pp 673–676

Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A, Messeguer J (2009) Effect of volunteers on maize gene flow. Transgenic Res 18:583–594

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O’Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. Agric Ecosyst Environ 84:1–20

Raybould A (2011) The bucket and the searchlight: formulating and testing risk hypotheses about the weediness and invasiveness potential of transgenic crops. Environ Biosafety Res 9:123–133

Raybould AF, Cooper JI (2005) Tiered tests to assess the environmental risk of fitness changes in hybrids between transgenic crops and wild relatives: the example of virus resistant *Brassica napus*. Environ Biosafety Res 4:125–140

Raybould AF, Gray AJ (1993) Genetically modified crops and hybridization with wild relatives: a UK perspective. J Appl Ecol 30:199–219

Raybould A, Tuttle A, Shore S, Stone T (2010) Environmental risk assessments for transgenic crops producing output trait enzymes. Transgenic Res 19:595–609

Ricroch AE, Bergé JB, Kuntz M (2011) Evaluation of genetically engineered crops using transcriptomic, proteomic, and metabolomic profiling techniques. Plant Physiol 155:1752–1761

Sanvido O, Romeis J, Bigler F (2007) Ecological impacts of genetically modified crops: ten years of field research and commercial cultivation. Adv Biochem Eng Biotechnol 107:235–278

Sjöberg L (2004) Principles of risk perception applied to gene technology. EMBO Rep 5:S47–S51

Turner AJ (1982) Soil survey of Willacy County Texas. United States Department of Agriculture Soil Conservation Service. Available at http://soildatamart.nrcs.usda.gov/manuscripts/TX489/0/Willacy.pdf

Wallace HA, Brown WL (1988) Corn and its early fathers. Iowa State University Press, Ames

Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, Gray A, Olin SS, Schiemann J, Sears M, Wu F (2010) Problem formulation in the environmental risk assessment of genetically modified plants. Transgenic Res 19:425–436