

The case of the monarch butterfly: a verdict is returned

Angharad M.R. Gatehouse, Natalie Ferry and
Romaan J.M. Raemaekers

A publication reporting the harmful effects on the monarch butterfly of maize genetically modified to express insecticidal δ -endotoxins from the soil bacterium *Bacillus thuringiensis* (*Bt*) caused much public interest. A series of ecologically based studies were subsequently carried out to evaluate rigorously the impact of pollen from such crops and to quantify the risks. The results demonstrated that the commercial large-scale cultivation of current *Bt*-maize hybrids did not pose a significant risk to the monarch population. Further studies also demonstrated that *Bt*-expressing crops posed little risk to other nontarget insects, including beneficial insects such as pollinators and natural enemies.

One of the major public concerns voiced in opposition to the introduction of genetically engineered crops is their potential impact on the environment. In Europe, this concern has resulted in serious setbacks for the AgBioTech industry. Among the different agricultural traits being engineered into crops are those for the control of insect pests. However, in addition to controlling pests (targets), there is the potential for such crops to affect nontarget insects, including beneficial insects, and thus have a negative effect on ecosystems.

In 2000, transgenic crops were grown on 44.3 million hectares globally. Of this, 23% was maize that had been genetically modified (GM) to express insecticidal δ -endotoxins from the soil bacterium *Bacillus thuringiensis* (*Bt*) to control the European corn borer (*Ostrinia nubilalis*) [1]. Financial losses as a consequence of this pest are in excess of US\$1 billion per annum in the USA alone. Furthermore, *B. thuringiensis* colonize and kill a large range of insects, with different strains of the bacterium being specific towards different insect orders. This specificity is primarily determined by the array of crystal (Cry) proteins that are produced during sporulation [2]. A summary of the major Cry proteins, together with their known toxicity, is given in Table 1; further details can be obtained from the *Bacillus* Stock Centre (<http://bacillus.biosci.ohio-state.edu>). *B. thuringiensis* has also been used as a biopesticide by organic farmers over the past four decades [3], and it is interesting to note that this use has failed to cause the level of public concern now being expressed over the growing of transgenic crops expressing this insecticidal protein.

Initial studies

Given the current level of public concern surrounding GM crops, it is not surprising that a publication reporting the harmful effects of pollen from transgenic maize towards the monarch butterfly (*Danaus plexippus*), a 'conservation flagship species' in the USA, caused so much public interest and was readily embraced by the media [4]. In their letter to *Nature*, Losey and colleagues claimed that both the survival and consumption rates of larvae fed milkweed leaves (natural host) dusted with *Bt*-expressing pollen were significantly reduced [4]. On the basis of this single laboratory experiment, the authors concluded that their results 'have profound implications for the conservation of monarch butterflies'. Rather than contributing to the GM debate in a positive way, this publication polarized it further. Scientists in the field were quick to question the validity of conclusions based on such preliminary data. For example, no attempt was made to quantify the pollen dose on the leaves or to determine whether these levels used in the laboratory assay reflected realistic levels to which the larvae would be exposed in the field. A further criticism levelled at the study referred to the fact that 'no-choice' tests were carried out. Although this scenario does not reflect the field situation, it could be argued that no-choice tests do provide the necessary baseline data on which subsequent studies would be designed.

A further study, published shortly after the *Nature* letter, made similar claims to Losey *et al.* [4], reporting that the effects of *Bt*-maize pollen on the monarch butterfly could be observed at least 10 m from transgenic field borders, although the highest larval mortality was likely to occur on milkweed plants within a range of 3 m from the edge of the maize field [5]. These studies were again laboratory based, but were more realistic in that the pollen levels used were comparable to those found in the field. Although these authors were more cautious in their conclusions, they recommended that the ecological effects of such transgenic crops required greater evaluation before being planted extensively.

In the wake of these two earlier reports, a series of ecologically based studies were commissioned to evaluate more rigorously the impact of pollen from the *Bt*-maize crop, and to quantify the risk posed by the commercial wide-scale growing of *Bt*-maize. Because different *Bt*-maize events (see Table 2) use different promoters to control gene expression, they will have different expression patterns for *Bt* proteins (Table 2). The findings from this comprehensive set of studies were recently published, and their significance is discussed below.

Quantifying the hazard

Risk is defined as 'hazard \times exposure'; thus, even if the potential hazard is great, if the exposure is effectively zero, so will be the risk. In an attempt to quantify the hazard, Hellmich *et al.* [6] carried out a systematic study to evaluate the toxicity of purified *Bt* protein to

Angharad M.R. Gatehouse*
Natalie Ferry
Romaan J.M. Raemaekers
Dept of Agricultural and
Environmental Science,
University of Newcastle,
Newcastle upon Tyne,
UK NE1 7RU.
*e-mail: a.m.r.gatehouse@
ncl.ac.uk

Table 1. Pesticidal activity of *Bacillus thuringiensis* crystal proteins

Insect order or organism	Cry protein
Lepidoptera	Cry1A, Cry1B, Cry1C, Cry1E, Cry1F, Cry1I, Cry1J, Cry1K, Cry2A, Cry9A, Cry9C, Cry9I, Cry15A
Coleoptera	Cry1I, Cry3A, Cry3B, Cry3C, Cry7A, Cry8A, Cry8B, Cry8C, Cry14A, Cry23A
Diptera	Cry2A, Cry4A, Cry10A, Cry11A, Cry11B, Cry16A, Cry19A, Cry20A, Cry21A
Hymenoptera	Cry22A
Nematodes	Cry5A, Cry6A, Cry6B, Cry12A, Cry13A, Cry14A
Liver fluke	Cry5A

monarch larvae. Of those δ -endotoxins tested, Cry9C and Cry1F were relatively nontoxic to first-instar larvae (the most susceptible stage), whereas the larvae were sensitive to Cry1Ac and Cry1Ab [6]; the fact that Cry1A proteins were toxic is not surprising because they were originally selected on the basis of their known toxicity to lepidopteran pests [7,8]. As expected, pollen from transgenic maize expressing Cry9C and Cry1F was nontoxic to monarch larvae. However, pollen from maize expressing Cry1Ab (events Bt11 and MON810) was also nontoxic; in both cases, the promoters used in the gene constructs were almost inactive in pollen, resulting in very low levels of the gene products accumulating. Thus, in the case of event Bt11 and event MON810, although there was hazard in that Cry1Ab is toxic to monarch larvae, there was minimal exposure, and thus negligible risk.

One line of transgenic maize expressing Cry1Ab, event 176, did produce pollen that did have deleterious effects on monarch larvae at high doses. Pollen from this line contained 40-fold higher concentrations of endotoxin compared with MON810, owing to the use of a different promoter. This result demonstrates that levels of *Bt* expression in the pollen are very important for subsequent toxicity of the transgenic maize pollen. Similar studies have been extended to include larvae of another nontarget herbivore, the swallowtail (*Papilio polyxenes*), demonstrating that larvae from both species were adversely affected by the high *Bt*-expressing pollen [9,10]. Interestingly, these field-based studies showed that mortality was not associated with proximity to *Bt*-maize but could have been due, at least in part, to predation [10]. No deleterious effects were observed with pollen from maize event MON810, either in the laboratory or in the field.

The level of exposure of monarch larvae to the *Bt*-expressing pollen is the other crucial component in the risk-assessment process. Because monarch larvae feed primarily on milkweed, Pleasants and co-workers

carried out detailed analyses of the density of maize pollen on milkweed leaves, both within maize fields and at different localities, to obtain reliable information on naturally occurring pollen density profiles [11]. As expected, exposure of pollen to monarch larvae was highest within the maize fields, rapidly declining away from the field boundary. Of importance was the finding that the pollen density on the upper leaves, where the egg masses are laid, was only 30–35% of that on middle leaves, and that the pollen densities were significantly higher around the leaf midrib, an area avoided by younger larvae. All of these findings suggest that, in reality, vulnerable first-instar larvae were less exposed to maize pollen on the preferred host plant than had been thought originally.

Oberhauser and colleagues made an important contribution to the debate by investigating both the spatial and temporal overlap of pollen production by maize plants during the larval stage of the monarch butterfly [12]. There was greater temporal overlap in the northern compared with the southern part of the summer breeding range. Thus, migration behaviour of the insect is an important consideration to be taken into account. Using a comprehensive set of data from many different geographical locations, Sears *et al.* [13] formalized an approach to risk assessment that integrates aspects of exposure to characterize the risk posed to monarchs from *Bt*-maize. On the basis of a two-year study, these authors concluded that the impact of *Bt*-maize pollen from current commercial hybrids on monarch butterfly populations was negligible. Maize lines derived from event 176, which could give cause for concern, are in the process of being withdrawn and represent <2% of the crop planted.

Arriving at a verdict

In contrast to the two early reports [4,5], the evidence to date leads to a conclusion that transgenic *Bt*-expressing maize plants will not have a detrimental impact on the monarch butterfly. Despite a >40% increase in planting of *Bt*-maize, the monarch population was estimated to have increased by 30% over the same duration, as indicated by the Monarch Watch website (<http://www.MonarchWatch.com>) and Ref. [14]. Contrary to media hype, the primary threat to the monarch population is loss of crucial winter habitats in southern California and central Mexico (as described by the website <http://www.bio.org>), rather than the commercial growing of *Bt*-maize. It has been suggested that agricultural practices such as weed control might also adversely affect the monarch population [10].

It is important to put the GM debate into perspective; all technology has the potential for risk, but the relevant question is whether the new technology poses more, or less, risk compared with current practice. In a comparative study, the pesticide λ -cyhalothrin had a dramatic effect on the survival and development of the monarch larvae in non-*Bt* fields [15]; the effects of the *Bt*-expressing pollen, however, were negligible. This result supports the view that GM technology has the

Table 2. *Bt*-expressing maize lines tested for insecticidal effects on monarch butterfly

Event ^a	<i>Bt</i>	Promoter	Company
Mon810	<i>Cry1Ab</i>	CaMV35s (constitutive expression)	Monsanto
Bt11	<i>Cry1Ab</i>	CaMV35s (constitutive expression)	Novartis
176	<i>Cry1Ab</i>	PEPC (expression in leaves) CDPK (expression in pollen)	Novartis

^aEvent refers to a specific construct and transformation event that has been crossed into different maize lines.

potential to contribute to the preservation of biodiversity relative to other management practices [9].

Public concerns expressed over the growing of *Bt*-maize are not only restricted to nontarget insects, such as the monarch butterfly, but also to the potential impact on beneficial insects, such as the natural enemies of pest insects (predators and parasitoids) and pollinators (e.g. bees). Extensive laboratory and field studies have been conducted to evaluate the effects of *Bt* proteins through the tritrophic interaction where the plant represents the first trophic level, the pest the second trophic level and the predator/parasitoid the third trophic level. Although detrimental effects of *Bt* Cry1Ab have been observed in lacewings (*Chrysoperla carnea*) when incorporated into artificial diet [16], exposure of the predator to the amounts offered would never occur under field conditions. In fact, Dutton *et al.* [17] showed recently that no effects on this predator occur when fed 'contaminated' prey from *Bt*-maize plants. These findings are consistent with most other studies carried out to date using a range of different predators and parasitoids [18]. Similarly, *Bt* protein (Cry1Ba) exhibits no toxicity towards pollinators such as honey bees (*Apis mellifera*) [19]. Interestingly, the same study reported that *B. thuringiensis* biopesticide preparations at comparable concentrations had a significantly deleterious effect upon both survival and food consumption of bees; however, such preparations will also contain other insecticidal compounds [2].

Implications for future developments

The large-scale cultivation of *Bt*-expressing crops raises wider issues than those discussed above. Of major concern, particularly to the organic farming community, is the potential for pest populations to develop resistance to *Bt* proteins. Such an event would not only limit the effective lifespan of *Bt*-expressing

crops, but would also limit *B. thuringiensis*-based biopesticides. Considerable effort has been devoted to delaying, what many consider as inevitable, the development of *Bt*-resistant pest populations [20,21]. A high-dose strategy coupled with the use of refugia has been recommended and adopted in most regions growing *Bt*-expressing crops. Another strategy being developed by the AgBioTech industry is to 'stack' or 'pyramid' genes encoding different Cry proteins; that is, use multiple resistance genes in a given line. Given that cross-resistance is known to occur for some *Bt* proteins [22], we feel that reliance upon this approach is somewhat short-sighted. Because industry has the responsibility to prolong the effective lifespan of *Bt* proteins, other strategies based on entirely independent and novel modes of activity should be developed and adopted.

Many lessons have been learnt from the 'monarch saga', not least that it is imperative that conclusions concerning environmental or nontarget effects of transgenic crops be based on appropriate methods of investigation and sound risk-assessment procedures. Although laboratory studies make a valuable contribution to risk assessment, they are limited in that they cannot replicate the diversity of environmental factors encountered in the field, nor can the impact upon communities be measured; longer-term field studies are thus essential to the risk-assessment process. For laboratory studies to be meaningful, realistic levels of test material must be used. Indeed, one of the reasons for the discrepancies between the original [4] and subsequent studies [6,9–13,15] appears to be due to the excessive and unrealistic levels of pollen used in the early bioassays [4]. On this, as with other issues, policy must be informed by rigorous and objective science. We, as scientists, have both the responsibility and duty to provide this.

References

- James, C. (2001) Global review of commercialised transgenic crops: 2000. ISAAA Briefs No. 21, Preview, ISAAA
- de Maagd, R.A. *et al.* (2001) How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. *Trends Genet.* 17, 193–199
- Flexner, J.L. and Belnavis, D.L. (1999) Microbial insecticides. In *Biological and Biotechnological Control of Insect Pests* (Rechcigl, J.E. and Rechcigl, N.A., eds), pp. 35–62, CRC Press
- Losey, J.E. *et al.* (1999) Transgenic pollen harms monarch larvae. *Nature* 399, 214
- Hansen-Jesse, L.C. and Obrycki, J.J. (2000) Field deposition of *Bt* transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia* 125, 241–248
- Hellmich, R.L. *et al.* (2001) Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11925–11930
- Peferoen, M. (1992) Engineering of insect-resistant plants with *Bacillus thuringiensis* crystal protein genes. In *Plant Genetic Manipulation for Crop Protection* (Gatehouse, A.M.R. *et al.*, eds), pp. 135–153, C.A.B. International
- Gatehouse, J.A. and Gatehouse A.M.R. (1999) Genetic engineering of plants for insect resistance. In *Biological and Biotechnological Control of Insect Pests* (Rechcigl, J.E. and Rechcigl, N.A., eds), pp. 211–241, CRC Press
- Wraight, C.L. *et al.* (2000) Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtail under field conditions. *Proc. Natl. Acad. Sci. U. S. A.* 97, 7700–7703
- Zangerl, A.R. *et al.* (2001) Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11908–11912
- Pleasants, J.M. *et al.* (2001) Corn pollen deposition on milkweeds in and near cornfields. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11919–11924
- Oberhauser, K.S. *et al.* (2001) Temporal and spatial overlap between monarch larvae and corn pollen. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11913–11918
- Sears, M.K. *et al.* (2001) Impact of *Bt* corn pollen on monarch butterfly populations: a risk assessment. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11937–11942
- Hodgson, J. (2000) Critics slam new monarch *Bt*-corn data criticized. *Nat. Biotechnol.* 18, 1030
- Stanley-Horn, D.E. *et al.* (2001) Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly in field studies. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11931–11936
- Hilbeck, A. *et al.* (1999) Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*. *Entomol. Exp. Appl.* 91, 305–316
- Dutton, A. *et al.* Uptake of *Bt*-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Environ. Entomol.* 27 (in press)
- Schuler, T.H. *et al.* (1999) Parasitoid behaviour and *Bt* plants. *Nature* 400, 825–826
- Malone, M.A. *et al.* (2001) Effects of a *Bacillus thuringiensis* toxin, two *Bacillus thuringiensis* biopesticide formulations, and a soybean trypsin inhibitor on honey bee (*Apis mellifera* L.) survival and food consumption. *Apidologie* 30, 465–473
- McGaughey, W.H. *et al.* (1998) *Bt* resistance management. *Nat. Biotechnol.* 16, 144–146
- Roush, R.T. and Shelton, A.M. (1997) Assessing the odds: the emergence of resistance to *Bt* transgenic plants. *Nat. Biotechnol.* 15, 816–817
- Tabashnik, B.E. *et al.* (1997) One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. *Proc. Natl. Acad. Sci. U. S. A.* 94, 1640–1644