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The monarch butterfly controversy: scientific interpretations of a phenomenon

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Summary

The future development and use of agricultural biotechnology has been challenged by two preliminary studies indicating potential risk to monarch butterfly populations by pollen from corn engineered to express proteins from *Bacillus thuringiensis*. Likewise, these studies have also challenged the way in which science should be performed, published in scientific journals and communicated to the public at large. Herein, we provide a history of the monarch controversy to date. We believe a retrospective view may be useful for providing insights into the proper roles and responsibilities of scientists, the media and public agencies and the consequences when they go awry.

Keywords: monarch butterfly, *Danaus plexippus*, biotechnology, Bt corn

The monarch butterfly controversy has polarized, rather than contributed to, discussion about the potential environmental impacts of using or not using genetically engineered plants in commercial agriculture. It has also raised issues about the quantity and quality of information included in scientific publications; the role of scientific journals in communicating information within the scientific community and to the public; and the nature of information required by public agencies to make decisions about using new technologies. Discussion of these important issues is warranted to ensure the integrity of the scientific process and enable it to be used as a basis for public policy decisions. In this paper we review the history of scientific communications regarding the monarch butterfly controversy. We are not aware of any single document in which this has been done, and we believe a retrospective view may be useful to the scientific and public communities. Our main objective is to reinforce an understanding of the roles and responsibilities of scientists, media, public agencies and those who oppose or advocate a specific technology, and to document the consequences when they go awry. Our roles in this phenomenon have been twofold. We have raised concerns about the quality of two published reports suggesting Bt corn pollen will cause significant harm to monarch butterfly populations under commercial field situations, and we have participated in more comprehensive studies to examine this issue. We have also served on a scientific advisory panel conducted by the US Environmental Protection Agency (EPA) to review the scientific information for registration renewal of plants expressing insecticidal proteins of *Bacillus thuringiensis*. These studies have included the effects of Bt plants on non-target organisms, insecticide-resistance management issues, and human health concerns.

Prior to the registration of insecticidal Bt corn plants (plants containing genes from the bacterium *B. thuringiensis*) that express proteins toxic to some insects, the EPA conducted risk assessments of the potential effects of Bt endotoxins on a wide range of organisms including birds, aquatic invertebrates, honey bees, ladybugs, earthworms, springtails, other non-target organisms and endangered species. In 1995, the EPA provided its opinion on the Ciba-Geigy application (Event 176) and stated ". . . the Agency can foresee no unreasonable adverse effects to humans, non-target organisms, or to the environment . . . ." (US EPA, 1995). At that time, EPA and scientists working in this area knew that endotoxins from *Btk* (*kurstaki*) were toxic to many Lepidoptera, such as the European corn borer (*Ostrinia nubilalis*) and other corn pests for which

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this product was developed, as well as some non-target organisms, including other Lepidoptera.

Exposure to Bt proteins by lepidopterous larvae was considered to be primarily due to ingestion of leaf tissue of Bt corn plants, and insects feeding on these plants would be considered pests. Another method of exposure to lepidopterous larvae would be through pollen deposits. Prior to registration of Bt corn, the amount of protein expressed in leaves, roots and pollen was documented (US EPA, 1995). Corn pollen is one of the heaviest wind-dispersed pollen grains, and a previous report (Raynor *et al*., 1972) stated that corn pollen tends to have limited movement out of the field, a fact later confirmed by Wraight *et al*. (2000). In its opinion in the section on endangered species, the EPA stated, "Although corn pollen containing Cry1A(b) k-endotoxin can drift out of corn fields, such pollen, at relatively very high dosages, was not toxic to the test species representative of organisms likely to be exposed to such pollen when corn plants containing the *cry1A(b)* gene are grown. The amount of pollen that would drift from these corn plants onto plants fed upon by endangered/threatened species would be very small compared to the levels fed to the test species" (US EPA, 1995). Although the EPA tested Bt corn or pollen on 'representative organisms' rather than all non-target organisms, we believe the EPA based its decision to register this product on sound science and well reasoned assumptions. However, its decision has come under criticism because of two reports of the monarch butterfly, *Danaus plexippus*, and Bt corn.

In an abstract for a poster presented at a North-Central branch meeting of the Entomological Society of America in March 1999, Hansen and Obrycki (1999) reported on a study conducted in 1998 in Iowa in which potted common milkweed plants, *Asclepias syriaca*, the host of the monarch butterfly, were placed within and at various distances from plots containing Bt and non-Bt corn. The potted plants were retrieved and examined for pollen deposition, and the leaves were assayed for the presence of Bt pollen by placing first-instar larvae on the leaves and allowing them to feed. As expected, pollen levels were highest on plants placed within the field, and dropped off sharply even 1 m from the field edge. Mortality of larvae feeding on leaves from plants within the field resulted in 16% (corrected) mortality. This initial report did not provide many details and was not subject to peer review and, because it was presented at a regional meeting, it was largely overlooked by scientists and the press.

In 1999 in New York, John Losey and colleagues conducted a laboratory study involving monarch butterfly larvae and Bt corn pollen. Their study consisted of depositing an unspecified amount of corn pollen from N4640 (a Bt hybrid producing Cry1Ab protein) or an unrelated, untransformed hybrid onto milkweed leaves and placing 3-day-old monarch larvae on the plants. They recorded leaf consumption, larval survival and final larval weight over a 4-day period. The authors found lower survival of larvae feeding on leaves dusted with Bt pollen compared with leaves dusted with untransformed pollen or on control leaves with no pollen. They also found reduced consumption of leaves dusted with Bt or untransformed pollen, compared with control leaves with no pollen. From these laboratory data, the authors developed a scenario in which they hypothesized that there could be "potentially profound implications for the conservation of monarch butterflies" (Losey *et al*., 1999) with the widespread use of Bt corn.

Prior to submission, Losey asked several people to review the article; the senior author of this paper was one of the reviewers who recommended against publication because of methodological problems, lack of field data and potential for misrepresentation of the study, but urged that a more careful study, including field aspects, be conducted to address the questions the authors were asking. The authors decided to publish their findings and, according to Knight (2000) in an interview with Losey, the article was submitted to and rejected by *Science* then resubmitted to *Nature*, which accepted it as a 'Scientific Correspondence'. Industry representatives learned of this potential paper, were concerned about its publication, and requested a meeting with Losey and his co-authors. In the meeting (attended by the senior author of this paper), industry personnel expressed their concerns about the methodology, including the lack of proper controls and missing details such as the dose used, and the potential for this study to be misinterpreted to the media and the public. The authors decided to go ahead and the article was published in May 1999 (Losey *et al*., 1999). Prior to its publication it was held under 'embargo', except that, according to Knight (2000), *Nature* did what it always does, it selected its hot story and fed it as an advance 'inside tip' to science writers. Once the article was released, it garnered the attention of the public and the scientific community and "knocked the biotech industry on its derriere" (Knight, 2000).

The choice of which scenario to use for determining the potential impact of agricultural biotechnology has a significant influence on the response of the audience. The monarch butterfly has fascinated generations of both public and scientific communities because of its beauty and complex biology (Monarch Watch, 2001). Each year, monarchs west of the Rocky Mountains travel to their overwintering sites along the California coast, while those east of the Rockies migrate to the mountains in Central Mexico. In all the world, no butterflies migrate like the monarchs of North America: they travel up to 3000 miles and are the only butterflies to make such a long two-way migration every year (Monarch Watch, 2001). The same adults migrate back toward their northern habitats in the spring, laying their eggs on milkweed and producing additional generations, the last of which migrates south again in the fall. No-one yet knows how this generation finds its way back to the same overwintering sites as its predecessors. The behavior and beauty of the monarch has led it to become the symbol of the complexity we call nature. For many, these reports on the monarch butterfly were seen as an example of agricultural biotechnology, specifically pollen from Bt corn, disrupting nature.

The first wave of publicity on the Losey *et al*. (1999) paper included articles in the major print media (e.g. *New York Times*, *Wall Street Journal*, *Time*), and radio and national television coverage. Publicity spread throughout the world rapidly and the public and policy-makers reacted in a startling fashion, including an immediate 10% drop in the stock value of one of the major producers of Bt corn (Monsanto); freezes on the approval process for Bt corn by the European Commission; and calls for a moratorium on further planting of Bt corn in the USA (Shelton and Roush, 1999). Losey expressed "surprise" at the coverage and reaction to this laboratory study (for a more thorough understanding of the motivation of scientists to publish this work, see Knight, 2000). Along with the publicity in the popular media, the scientific community also responded quickly to this report. A discussion at the European Plant Biotechnology Network's Phytosfere meeting held in Rome soon after the *Nature* paper was published "indicated that the majority of delegates resoundingly rejected the work's validity", and one delegate reported that if he had used such methods in a study he "would expect to be chopped into little pieces during peer review" (Hodgson, 1999). We also concluded the study was weak because of the unspecified dose of pollen used and the unspecified endotoxin concentration in the pollen; the lack of a choice test; the use of inappropriate controls; and the lack of information on the potential for a temporal and spatial overlap of pollen shed, milkweed plants and monarchs under natural field conditions. Other scientists familiar with corn-pest management expressed concern about the methodology as well as "the emerging trend toward publishing little laboratory studies . . . (which) cause big problems for scientific credibility . . ." (Foster, 1999). While the scientific culture encourages debate about research findings, there was something different about the level of discussion and passion on the monarch controversy, and this seemed due, in part, to the monarch being a symbol of nature, to the novelty of biotechnology, and to changes in modern communication.

Scientists and the biotechnology industry responded quickly to the scientific and public interest in alleged threats to the monarch butterfly. Industry worked with United States Department of Agriculture (USDA) and university scientists in entomology and weed science to form a consortium of researchers to help develop a comprehensive research program, and distributed more than US$100 000 in 1999 to researchers from six universities to assess the potential risk to monarch populations from Bt corn pollen under natural environmental conditions. These studies focused on determining the potential effects of Bt pollen on monarch larval growth and survival (hazard), and evaluating the probability that larvae would consume Bt pollen (exposure). The panel urged the public not to over-react to the initial study reports, but to allow the scientific community to complete a thorough, science-based risk assessment.

The Losey *et al*. (1999) paper might have been the first salvo in the brewing battle about the potential environmental impacts of agricultural biotechnology, but it was not the last. In July 2000, researchers in Illinois published a paper showing the absence of toxicity of Bt corn pollen on another common and beautiful butterfly, the black swallowtail, *Papilio polyxenes*, under field conditions (Wraight *et al*., 2000). In their study they used potted wild parsnip plants, a host for the black swallowtail, infesting them with early-instar larvae and placing them in an array along the edge of a field of Bt corn containing the Cry1Ab endotoxin. This study was perceived by many to be more realistic than Hansen and Obrycki's (1999) report because it examined mortality factors under field conditions where environmental factors such as wind, sunlight and moisture may influence the deposition of pollen and its consumption by larvae. The authors found no relationship between mortality of the larvae and proximity to the field or pollen deposition on the host plants. In laboratory assays, the authors also failed to see mortality even at doses fivefold higher than the highest pollen density seen in the field. The authors cautioned that their study does not indicate that monarch butterflies will also be unaffected, but they urged that "field studies as well as appropriately controlled laboratory studies are necessary before such a conclusion can be drawn."

The Wraight *et al*. (2000) report, while certainly not receiving the same level of press attention as the Losey *et al*. (1999) report, became the focus of series of e-mails on a listserve (Ammann, 2000a) between one of its authors, May Berenbaum, and Jane Rissler from the Union of Concerned Scientists, as well as an outlet for others who wished to express their opinions on the monarch controversy. To illustrate our present age of communication by electronic means, and how it can rapidly contribute to the controversy within the scientific and public communities, a series of e-mail correspondence is summarized. Rissler claimed the Wraight *et al*. (2000) experiment was "faulty in experimental design", to which Berenbaum replied that "equally strict standards are not being applied to studies with outcomes that are more consistent with a particular world-view", referring to the

Losey *et al*. (1999) article. In that same e-mail, Barry Palewitz noted that he had investigated the Bt corn line (NK 4640) used in the Losey *et al*. (1999) laboratory test and found it to be a Northrup King variety which used Monsanto's YieldGard Bt. Palevitz stated the corn used was actually event Bt 11, which has "toxin levels in pollen so low as to be at the limit of detectability, according to Monsanto. That's one of the reasons why the Losey *et al*. (1999) paper is strange. They must have used A LOT (*sic*) of pollen to get the effect they observed, which also opens it up to non-specific action." The scientific community, and anyone else who had subscribed to Ammann's popular listserve, was seeing a public and passionate debate about science, regulatory processes and other deeply held feelings, and this debate was not confined to meetings or letters to the editor but was now occurring in 'real time'.

One month after the Wraight *et al*. (2000) paper was published, the scientific community was able to review the full study that Hansen and Obrycki (2000) had presented as a poster presentation in March 1999. In their paper, they claimed that they presented the first evidence that transgenic Bt corn pollen "naturally" deposited in a corn field "causes significant mortality" of monarch larvae. Unlike the Losey *et al*. (1999) report, they compared two Bt lines with their genetically similar lines, and quantified the pollen levels deposited on the leaves of the potted milkweed plants. Of the Bt lines used, one (event 176) is known to have a much higher concentration of Bt endotoxin in the pollen (>50 times higher) than the other Bt line used (event Bt11) (US EPA, 2000). While the authors indicate their study was a field study, others thought differently, as only the collection of the leaf samples with the pollen itself was carried out in the field while exposure was measured under laboratory conditions (Ammann, 2000b). This study also made the assumptions that the placement of the potted plants simulates the natural distribution of milkweed plants in and near corn fields; and that adult monarchs would find and lay their eggs on these plants. These assumptions, as well as the assumption that monarch populations occur at the same time as pollen shed, go to the heart of the matter of whether monarch larvae will be exposed to lethal concentrations of natural deposits of Bt pollen.

Criticism of the study also focused on the bioassays. As the authors noted in their paper, there were large discrepancies between the toxin levels in pollen that they measured and those from replicated measurements accepted by the EPA. For example, for Bt 11 corn their level was over four times higher (0.39 versus 0.09 $\mu g\ g^{-1}$). and for event 176 corn their toxin levels were less than four times lower than the EPA-accepted figures. Furthermore, they detected Bt-toxin concentrations of 0.052 $\mu g\ g^{-1}$ pollen, half the accepted figure for Bt 11, in pollen collected from the 'non-Bt' variety 4494. The authors reported that, due to the methods they employed in extracting pollen from corn tassels, the resulting pollen samples contained 43% of plant debris. The impact of this debris has been shown by Hellmich, Siegfried, Sears, Stanley-Horn, Mattila, Spences, Bidne and Lewis (unpublished results) to cause significant mortality, and reduce weight gain by >80%, of larvae exposed to contaminated versus uncontaminanted pollen samples. Such debris is an artifact of the collection method. Thus it was not surprising that high doses of this non-Bt variety caused the same mortality to larvae as high doses of event Bt 11 transgenic corn. Furthermore, it was odd that there was 40% survival of larvae exposed to event Bt 11 pollen at doses of both 135 and 1300 grains cm^{-2}. These questions about the study, along with the fact that event 176, which has a higher concentration of Bt in the pollen, constitutes <2% of the corn acreage planted in 2000, did not seem to enter the public discussion of this report. However, on the day the report was published Obrycki received 44 calls from the press (Abbott, 2000).

Prior to the Hansen and Obrycki (2000) article, in December 1999 the EPA issued a monarch butterfly data call-in notice to the registrants of Bt corn. This action was engendered by reactions from some media and antibiotechnology groups to the Losey *et al*. (1999) paper and the Hansen and Obrycki (1999) poster. The data call-in requested information on five areas: the distribution of monarch butterflies, milkweed plants and corn; corn-pollen release and distribution in the environment; toxicity of Bt corn Cry proteins and Bt corn pollen; monarch egg laying and feeding behavior; and monarch population monitoring (US EPA, 2000). Research by the scientific community to address these questions, with support from industry, was already under way. All available data were summarized and provided as a preliminary response to the EPA in March 2000 (US EPA, 2000). In 2000, additional studies were co-sponsored by the USDA and industry, with distribution of nearly US$200 000 in funds to university and government researchers co-ordinated by the USDA. These studies focused on confirming preliminary 1999 findings and on evaluating monarch survival and occurrence in corn fields under typical midwestern and eastern growing conditions. Some results of this research have been presented in several venues at meetings of the Entomological Society of America in 1999 and 2000, at a November 1999 monarch meeting in Chicago, and a USDA-sponsored workshop in February 2000. In October 2000, the EPA conducted a Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP) to examine the sets of scientific issues being considered by the EPA for the re-registration of Bt plants. The panel was asked to comment on the EPA's analysis of the currently available data on the potential impacts of three corn lines (MON810, Bt 11 and CBH351) on monarch butterflies (event 176 was being discontinued, primarily because of concerns about resistance management).

The findings of the panel are available online (US EPA, 2001). At the SAP meeting, new information was presented on field studies conducted in Maryland, Iowa and Ontario, Canada with the corn lines MON810 and Bt 11, but the entire panel did not have these data beforehand so could not thoroughly evaluate the data. Those who did present the data, including the junior author on this paper, stated that naturally deposited pollen from these two corn lines did not seriously affect monarch populations. Furthermore, laboratory bioassays with Bt pollen densities about 10 times higher than densities typically found inside corn fields showed no significant effects on monarch growth and survival. Others reported on aspects of monarch phenology and pollen shed in Minnesota, Iowa, Maryland and Ontario, Canada. Their data indicated that adult monarchs use milkweed in corn fields as ovipositional sites more than was first believed, and that there was variation between synchrony of monarchs and pollen shed in these states; however, some overlap of pollen shed and the occurrence of monarchs was observed at all locations. Despite such overlap, those who presented field and laboratory data on the dose of Bt pollen that populations of monarchs would be exposed to under field conditions stated that these two corn lines did not significantly affect monarch larval mortality and development relative to non-Bt corn. These important comprehensive studies are now being submitted for publication in peer-reviewed journals, and their conclusions indicate that the impact of Bt corn pollen on monarchs in the field has been negligible.

Decisions on the future of Bt plants depend on federal agencies and on public opinion. It is important to note that the EPA (2001) SAP states that "Sound science findings must drive decisions by the Agency. Inappropriate conclusions drawn from an insufficient database are to be avoided in this process." This quote begs the question about what is a sufficient database. At what stage in a scientific investigation is it appropriate to publish so that one will be confident in one's conclusions and can present them with balance? How can our major scientific journals contribute best to helping the public understand complex issues such as biotechnology and the risks of using or not using it? We believe these are very important questions facing science today, and they need to be addressed to ensure the integrity of the scientific process. The public, and even the scientific community, should see the monarch debate in a larger framework. Science is still done by humans who approach their subject from different perspectives and prejudices, and who are subject to the real or imagined pressures of their peers and institutions. How scientists communicate among themselves and to the public, and how public policy will be formed, are being challenged by the monarch/Bt corn case.

In a world made smaller by our modern communication methods, more care is needed – misinformation or partial information can easily influence public policy (Shelton and Roush, 2000) and "both scientists and the public must take note and move forward with even more selfless integrity" (Shelton and Roush, 1999). Scientists need to be heard, but the reporting of biotech issues has changed markedly since 1997 and "moved from being a scientific issue to being a social issue" (Abbott, 2001). The media coverage has exploded, and the sources of information have changed. As noted by Abbott (2001), in late 1999 the *New York Times* was running "almost one article per day on this (biotech) topic". In the media coverage of GMOs in England and the US from 1997 to 2000, Abbott found that the *New York Times* and the London *Times* were using scientists less and less as sources for stories, and by September 2000 only 12% of the news stories quoted university scientists. In contrast, Abbott notes that environmental activist groups such as Greenpeace, the Environmental Defense Fund and the Union of Concerned Scientists were increasingly used as sources of news, and that the newspapers reviewed were more than twice as likely to use a quote from one of these sources than from university scientists.

The monarch has become the symbol of the anti-GMO movement. In early 2001, the Environmental Defense Fund sent its fund-raising letter with the following opening remarks: "Each year, millions of black and orange Monarch butterflies migrate thousands of miles from Mexico to Canada . . . but new scientific evidence suggests that the milkweed Monarch caterpillars are eating may kill them and unless we act now, there could be more unpleasant 'genetic surprises' in the near future." Similarly, the January/February issue of the magazine of the Sierra Club, *Sierra*, has a postcard in it addressed to the CEO of Kraft Foods asking them to "move to GE-free production". On the other side of the card is a collage of Monarch butterflies with the words, "Genetically engineered food - even butterflies find it hard to swallow". We find these 'statements' irresponsible from a scientific viewpoint, and believe they were created, in part, due to a rush to publish reports that were incomplete and misleading. Furthermore, we believe that such a focus on the monarch situation has caused some scientists to shift their research focus away from more pressing and worthy aspects of agricultural research.

We find the monarch controversy frustrating and frightening in the quest to improve plant protection practices. As field-oriented entomologists involved in integrated pest management, we have seen environmental problems associated with managing insects with conventional insecticides, including their effects on non-target organisms. Microbial insecticides, such as Bt, have been around for decades but are not widely used because they have not provided the level of control required at a reasonable cost. In fact, despite Bt being available for >40 years as a foliar insecticide, it accounts for <2% of all insecticides. It was only when the genes for production of Bt proteins were engineered into plants that Bt became a major insecticide. Bt plants were grown on 11.5 million ha in 2000 (James, 2000). Of the US$8.1 billion spent annually on all insecticides worldwide, it has been estimated that nearly US$2.7 billion could be substituted with Bt biotechnology applications (Krattiger, 1997). Thus what once was only a minor insecticide when applied to a plant's foliage is now a major insecticide.

As Bt crops are being deployed, we are learning about the risks and benefits of this new technology, and are in a better position to evaluate them in comparison with the risks and benefits of alternative technologies. The EPA estimates that the use of Bt crops in the USA results in an annual reduction of >7.7 million 'acre treatments' of synthetic insecticides (US EPA, 2000), mostly broad-spectrum insecticides which can affect non-target organisms and potentially lead to environmental and human health risks. Scientists may be more comfortable than the general public in comparing risks of technologies. In the case of the monarch butterfly, many entomologists believe the risks of Bt corn pollen are small compared to the destruction of their overwintering habitats (Reuters, 2001). But in the highly publicized and highly polarized discussions of Bt plants, the monarch has become a symbol and seems to have taken on a life of its own, a life much larger than the laboratory study which placed it squarely in the center of the controversy. As with any technology, there will be questions raised about the potential environmental effects of transgenic plants, but these concerns should be addressed in a rigorous scientific fashion unencumbered by sensationalism. We hope that scientists, the media, public policy-makers and the general public will regain their rightful roles in the dialogue about agricultural biotechnology, and be able to view the forthcoming data on field studies of monarchs and Bt corn in a more thoughtful and reserved manner than was observed with the previous two reports on monarch butterflies and Bt corn.

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