

Blog: Debate Contribution from Sept. 5, 2000 Klaus Ammann

Dear friends,

this is the most thorough answer to the Monarch paper of Hansen / Obrycki I have seen so far.

It has been circulated by the foodbiotechnet@listserve.health.org and contains some new elements of critique.

Here the link, where you can get the study in pdf format:

<ftp://debate.friends@sgiserv.unibe.ch/home/debate/OEC.pdf>

see also Debates:

2000'0611 a, 2000'0821 a (the study), 2000'0823 a,
2000'0824 a, 2000'0825 b, 2000'0828 a

Klaus

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selected Summary

The results of these experiments do not represent the potential impact of Bt pollen from all events, as the authors suggest, across the extensive range of the monarch butterfly in North America. We await the results of the comprehensive international study which includes studies conducted over a two year period (1999-2000) in the field and whose preliminary results,

already presented at several scientific meetings, provide a much more complete and balanced assessment which indicates that Bt corn does not pose a significant threat to Monarch populations as implied in this study or as portrayed in some of the press.

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Following are comments from Mark Sears, PhD of the University of Guelph and Anthony Shelton, PhD of Cornell University regarding the latest published research studying the effects of Bt corn pollen on Monarch butterflies.

Questionable Conclusions From Latest Monarch Study

By MARK SEARS, Ph.D.

University of Guelph

ANTHONY SHELTON, Ph.D.

Cornell University

A recent paper titled, "Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly", by Laura Hansen and John Obrycki from Iowa State University, was published online in the European journal *Oecologia*. It has been described as the first field study which describes the effect of certain Bt corn pollen deposits on milkweed leaves and their effects on larvae of the monarch butterfly. This report has received considerable media attention and we believe it is important to offer some comments.

We believe careful examination of the methods, conclusions and discussion of this paper are warranted, and that such examination raises serious questions about what, if any, conclusions can be realistically drawn from this study. As field biologists we realize the difficulty in conducting quality field research relevant to real world situations, but we believe this study does little to help understand potential risks of deploying Bt plants in the field.

In order to assess the deposition of pollen in the field, the authors placed potted milkweed plants in or at various distances from the edge of corn fields during pollen shed. Samples of milkweed leaves were brought to the laboratory and newly hatched larvae of Monarch butterflies were placed on leaf disks cut from the leaves and fed for 48 hours. While we appreciate the difficulty of setting up these tests, these methods make the assumptions that the placement of the potted plants simulates the natural distribution of 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 depositions of Bt pollen. Furthermore, we are aware of work indicating that Monarch butterflies avoid laying their eggs on leaf tissue with high deposits of pollen. These factors, along with several others, make us question the "field" aspect of this paper.

In order to assess the dose of pollen required to cause mortality of newly hatched monarch larvae, another set of experiments was conducted. Pollen was collected from the field and applied to leaves in the laboratory at three different doses (14, 135 and 1300 grains per square centimeter). Small larvae were placed on disks and fed for 48 hrs. Mortality and development and growth characteristics were assessed. The point is, only the collection of leaf samples with pollen and the collection of the pollen itself was carried out in the field.

Field trials are those that are conducted entirely under the conditions prevalent in the field during the experimental period. Because of this, the results will be affected by factors such as moisture on the leaf surfaces, variable temperature and humidity, degradation of the pollen by sunlight, moisture, microorganisms, rainfall, wind, natural dispersal and behavior of monarch larvae, predation on monarch larvae, and a host of other ecological factors, not to mention the adult Monarch choosing whether or not to lay her eggs on a pollen-infested plant. Some or all of these factors will have a direct result on the measurement of mortality and may completely overshadow the effects of the Cry 1Ab toxin expressed in the pollen. True field trials are necessary to understand the nature of pollen deposition on milkweed plants and the possible effect on monarch larvae or any other species of caterpillars.

Field trials are underway in an international comprehensive program involving scientists from Maryland, Iowa, Nebraska, Ontario, Canada and other places and the first year results have been discussed at several scientific meetings. The data from the comprehensive effort provide information on many of the important questions not addressed in this study such as whether natural populations of monarch are actually exposed to lethal effects of Bt corn pollen in the field. We believe great caution should be exercised in assessing the Hansen and Obrycki paper until publication of the scientific data from the international programs efforts, which we hope will be done in a timely fashion. But for now we must address our concerns with the present study.

A misleading statement by the authors and media implied that the results implicate all Bt corn types (or events expressing Cry 1Ab toxin). In the study, only pollen from Event 176 Bt corn showed any consistent lethal effect. Event 176 Bt corn represented about 2% of the total Bt corn acreage planted in North America in 1999 and probably is no more than 1% of the acreage in 2000. The pollen from Event 176 is considerably more toxic than pollen from Bt11 or MON810, the leading Bt corn products. It is misleading to imply that toxicity associated with that product is relevant to Bt hybrids planted by the vast majority of farmers.

The conclusions of the authors go far beyond the extent of the data presented. They imply in their discussion that significant amounts of pollen could be distributed within and up to 10 meters outside of cornfields such that significant mortality to monarch larvae would occur. Their own data do not support this speculation. Nowhere have they reported on the density of milkweed plants in or around fields or in different habitats in the area, nor have they provided any information on the phenology of the monarch populations in relation to the pollen shed period of the Bt corn hybrids. These are the points being addressed in the international comprehensive program. Without this information, it is improper to speculate on the risk associated with the Cry 1Ab toxin found in some Bt corn hybrids.

A more detailed discussion of study findings and procedures, some of which raise questions, follows:

1. Toxicity results are not consistent with those of other studies. Other researchers have found that pollen from Event Bt11 (and other events with similar expression levels such as MON 810), is not toxic to Monarch larvae at a dose of 150 grains per square centimeter or less, yet the Hansen and Obrycki study found mortality at 135 grains per square centimeter. A possible reason for this may be that the pollen used in the laboratory feeding studies was contaminated. The authors found that the pollen they collected from Bt11 plants contained 4.3 times more toxin than had been seen in previous replicated studies accepted by the Environmental Protection Agency (0.39 micrograms per gram at Iowa State vs. 0.09 micrograms in EPA-accepted data). The authors acknowledge that the collected pollen may have been contaminated with anthers, a part of the corn plant that retains pollen. Likewise, the toxin concentration in Event 176 was calculated to be several times less toxic than previous replicated research had shown. In addition, the pollen from non-Bt plants was found to contain trace levels of Bt. The authors acknowledge that it may have been contaminated during collection. These incongruities raise questions about the purity of the tested material.

2. The sample size of larvae was small. With most biological assays of this nature, usually hundreds of larvae are evaluated to give a realistic sampling. Only 35 larvae were exposed to leaf sections containing pollen from Event 176 actually taken from the field. Of the 35 exposed larvae, 20 percent (7 larvae) died. Given the small sample population, other factors could have contributed to mortality, such as handling and transport. In fact, the authors acknowledge that there was no correlation between dose and mortality. The leaf sections contained doses varying from 10 grains of pollen per square centimeter up to 306 grains. One would expect that insects exposed to the highest concentrations would have the highest mortality rate. This apparently was not the case, leading to the possibility that at least some mortality was linked to other factors. In the experiment where larvae fed on leaves dosed with 3 levels of pollen, the sample sizes were also small (10 and 16 larvae per treatment).

3. The concentrations that caused toxicity were not representative of field concentrations they report. The larvae were exposed to three selected concentrations - 1300 grains of pollen per square centimeter of milkweed leaf surface, 135 grains per square centimeter, and 14 grains per square centimeter. No adverse effect was seen at the lowest dose. While significant mortality was seen at the middle dose and the highest doses, the actual field sampling showed that these doses were rarely seen near or even within the cornfields. The authors predict that concentrations of 1300 grains per square centimeter could be seen within fields, but their survey showed a range of 0 to 152 grains within the field. The average concentration of Bt11

within the cornfield was 74.2 grains in 1998 and 115 grains in 1999.

4. Larvae were exposed to very small sections of leaf and had no choice of diet. The authors collected milkweed leaves from the field, and then they cut out small disks (0.79 square centimeters). The disks were placed on a moistened filter paper in a petri dish. One neonate (first instar) larva was placed on the top of each disk for 48 hours. This is a very artificial environment. In the wild, larvae would most likely hatch on the underneath side of a leaf, sheltered from any pollen that may have collected on the upper surface. They also would be free to move across the entire leaf surface (about 82 square centimeters per side) or even to move to other leaves or adjoining plants. They would not have to eat pollen. Other research has shown that larvae tend to avoid pollen of any type. Other observations have shown that pollen on milkweed leaves congregates in vein depressions and folds, leaving most of the leaf surface unexposed.

5. Older larvae that were exposed to concentrations in the laboratory had much higher survivorship than neonate larvae. Two studies were conducted; one used larvae less than 12 hours old and the other used larvae 12 to 36 hours old. At the rarely seen dose of 135 grains per square centimeter, only 30 percent of young larvae exposed to Event 176 survived, but 63 percent of older larvae survived. For Bt11, the survival was 40 percent for young and 75 percent for older. This is a significant finding, because Monarchs tend to lay their eggs on the underside of leaves, sheltered from pollen. The neonate larvae would most likely spend their first 12 hours feeding on the underside of the leaf.

6. Survival rates at various doses raise questions. The study showed a 40 percent survival rate for larvae exposed to Bt11 at 1,300 grains as well as 135 grains. It is very unusual that a dose 10 times lower would produce the same effect. It is also interesting that larvae exposed to 1,300 grains of non-Bt pollen had a 40 percent survival rate, the same as those exposed to 1,300 grains of Bt11.

7. Larvae that survived exposure developed into normal adults. There was no difference between total development times for exposed and unexposed larvae. Pupal weights, adult dry weights, lipid weights and forewing lengths were also similar among exposed and unexposed insects.

8. We also would like comment on what we think is an important point in the paper but which has not received adequate comment, i.e. what are the risks and benefits of Bt corn compared to alternative methods of control of the European corn borer? We believe this is the crux of the argument about the future of Bt crops, and question a major point made in the last paragraph of the paper. The authors are trying to make the point that the comparison of the safety of transgenic crops like Bt corn may not be "appropriate if

previous insecticide use against the target pest was low". Their claim that in 1995 only "2.2% of the corn in Iowa was treated with broad-spectrum insecticides for control of the European corn borer" is a bit of a misleading snap shot. Marlin Rice, an experienced corn entomologist who is the same Department as the authors, was recently quoted in the press as stating from a recent survey of corn growers that "27 percent of (corn growers) wanted (Bt corn) to eliminate the need for insecticides to control the pest (corn borers)". In addition, foliar sprays of insecticides do not provide maximum control since timing is crucial and because it is difficult to get the insecticide to the target pest, especially when it is applied by air. Such concerns are not issues in Bt corn. For this reason, the comparison the authors try to make is also misleading.

The examples given above illustrate that the study does not duplicate a field environment, and they raise questions about whether the tested material was more toxic than pollen that would occur in the field. The lack of a dose response is particularly troubling, as is the small sample size. The study does confirm what has been seen in various field studies - that pollen density drops off rapidly at the edge of a cornfield.

The results of these experiments do not represent the potential impact of Bt pollen from all events, as the authors suggest, across the extensive range of the monarch butterfly in North America. We await the results of the comprehensive international study which includes studies conducted over a two year period (1999-2000) in the field and whose preliminary results, already presented at several scientific meetings, provide a much more complete and balanced assessment which indicates that Bt corn does not pose a significant threat to Monarch populations as implied in this study or as portrayed in some of the press.

Mark Sears, Ph.D. ` Anthony Shelton, Ph.D.
 Professor of Entomology Professor of Entomology
 University of Guelph NYSAES, Cornell University
 Guelph, Ontario, Canada Geneva, NY 14456
 MSEARS@evbhort.uoguelph.ca ams5@cornell.edu
 519-824-4120 315 787 2352

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