

Bt-maize and non-target organisms

Compilation of available data



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1. Summary

Bt maize represents a new, environmentally friendly way to control devastating insect pests and ensures yield to the farmer.

Novartis has developed two different types of genetically modified maize plants containing the truncated CryIA(b) protein. CryIA(b) is an endotoxin with highly specific insecticidal effects on certain Lepidopterans and protects the maize plants against corn borer larvae. The primary target insect is the European corn borer (*Ostrinia nubilalis*) and the pink stem borer (*Sesamia spp.*), devastating maize pests that, when left uncontrolled, cause an estimated \$1 billion in lost U.S. corn yields annually. Some types of Bt maize also provide protection against other Lepidopteran pests that feed on maize, including the Southwestern corn borer, corn earworm and fall armyworm. Non-target insects are those that *do not* feed on maize, including other Lepidopterans such as the Monarch butterfly.

Novartis Seeds stands by the quality and safety of its Bt maize. Extensive and rigorous studies, undertaken for review by regulatory authorities and other bodies over the past years have concluded that Bt maize is as safe as conventional maize. These results have been confirmed by more than 30 scientific committees.

The overwhelming body of scientific evidence published so far supports the view that non-target species (including beneficial insects) are unaffected by the presence of Bt maize in the fields.

This compilation of data gives an overview of the field and laboratory studies that were conducted or are currently ongoing.

To get a quick overview, the organisms examined and the results of the various studies, as well as the corresponding authors are given in a summary table on the following pages (5-7).

Novartis has conducted several studies with third parties to monitor insect populations associated with maize cultivation since 1993, in the context of hybrid registration in Spain and France. In a continuing effort to monitor the effect of Bt maize on the environment, Novartis is planning a monitoring programme which looks at the impact of Bt maize on insect populations, among other parameters. Novartis is evaluating research protocols to deliver data that can provide answers to the theoretical issues raised by individual laboratory studies.

Additional field trials have compared the populations of insects in plots of Bt maize and non-Bt maize, as well as the impact of a conventional chemical insecticide commonly used on maize. Results of these studies indicated no difference in the number of total insects or the numbers in each of the specific groups, such as Coleoptera (e.g., lady beetles), Homoptera (e.g., aphids) and Hymenoptera (e.g. bees). No effects on Diptera, Lycosidae, Linyphiidae, Opiliones, Staphylinidae, Carabidae, Cicadellidae, Thysanoptera, Anthocoridae, Nabidae, Coccinellidae, Chrysopidae and Chalcoidea could be found. In contrast, treatment with the conventional insecticide had dramatic effects on the total numbers of insects and on the numbers within specific groups, including beneficial insects, compared to the untreated plots. No effect of the Bt protein was detected on birds, broiler chickens, rodents and small mammals.

These studies found no evidence that exposure to Bt maize protein expressed in maize plants or pollen resulted in any toxic effect on the organisms tested. To the contrary, due to its selective toxicity to specific Lepidopteran pests (e.g., European corn borers), Bt maize will help preserve populations of beneficial insects that might otherwise be threatened by use of chemical insecticides.

Summary table of studies conducted to assess the effect of Bt- Maize on non-target organisms

Organism studied	CryIA(b) protein applied as	Study design	Result	Authors	Page
Daphnia magna	Pollen from Bt-176 maize and isogenic controls	Laboratory study	No effect, daphnids of all groups normal	Springborn Laboratories*	Study 1 Page 11
Earthworms (<i>Eisenia foetida</i>)	CryIA(b) protein preparation from Bt-176 plant leaves	Laboratory study	No effects on survival or signs of toxicity	Springborn Laboratories*	Study 2 Page 11
Lady beetle (<i>Coleomegilla maculata</i>) development from first instar larvae to adult	Pollen from Bt-176 maize and isogenic controls or pea aphids as optimal control diet	Laboratory study	Lower mortality rate of larvae fed on Bt-pollen (decrease of vitality for more than 50% compared to optimal diet in both groups)	Iowa State University*	Study 3 Page 12
Lady beetle (<i>Coleomegilla maculata</i>)	Pollen from Bt-176 maize and isogenic controls , supplemented with pea aphids	Laboratory study	No statistically significant differences in survival or development time	Pilcher <i>et al</i>	Study 3A, Page 13
Larval honeybee (<i>Apis mellifera</i> L.) development	Bt-176 maize pollen and a drop of water were administered as sole source of food	Laboratory study	No differences in the average number of days to emergence, nor were any behavioural effects observed	California Agricultural Research*	Study 4 Page 13
Larval honeybee (<i>Apis mellifera</i> L.) development	Pollen supply from whole transgenic maize plant	Semi-field study	Study ongoing	Novartis	Study 4A Page 14
Springtails (Collembola, <i>Folsomia candida</i> ,)	CryIA(b)-enriched maize leaf protein of Bt-176 maize	Laboratory study	Maximum acceptable toxicant concentration (MATC) range is 125 mg protein/kg soil [0.088 ppm CryIA(b)] to 250 mg protein/kg soil [0.175 ppm CryIA(b)]	Springborn Laboratories*	Study 5 Page 14
Several non-target insect populations: Diptera, Hymenoptera, and Coleoptera (Coccinellid family) as well as Homopterans	Whole transgenic Bt-176 maize plant	Field study	Results of the monitoring study indicated no difference in the number of total insects or the numbers in specific Orders	Ciba Seeds	Study 6 Page 15
Several non-target insect populations: Diptera, Hymenoptera, Coleoptera and Lepidoptera	Whole transgenic Bt-176 maize plant	Field study	No significant differences	Novartis	Study 7 Page 16
Aphids (<i>Rhodopalosiphum padi</i>) and green lacewings (<i>Chrysoperla carnea</i>)	Whole transgenic Bt-176 maize plant	2 year field study	No effect on aphids or lacewings reared on them	Lozzia <i>et al</i>	Study 8 Page 16/ Study 4 Page 24

*Studies sponsored by Novartis

Summary table of studies conducted to assess the effect of Bt- Maize on non-target organisms

Organism studied	CryIA(b) protein applied as	Study design	Result	Authors	Page
Natural enemy populations of European corn borer	Whole transgenic Bt-176 maize plant	Field study	No impact on egg mass predation, parasitism of egg masses and sloughing of eggs of the European corn borer; no difference in densities of predators	Orr <i>et al</i>	Study 9 Page 17
Lycosidae, Linyphiidae, Opiliones, Staphylinidae, Carabidae, Cicadellidae, Thysanoptera, Anthocoridae, Nabidae, Coccinellidae, Chrysopidae and Chalcoidea	Whole transgenic Bt-176 maize plant	Field study	Neither reduction in quantity, nor any changes in population development in any of the parasitic or predator species examined.	Candolfi <i>et al</i> *	Study 10 Page 17
Secondary lepidopteran pests (Lepidoptera: Noctuidae)	Bt-176 varieties, whole plants	Field study and laboratory study	No effects on <i>A. ipsilon</i> and <i>P. nebris</i> . <i>Pseudaletia unipuncta</i> (Haworth) reared on Bt- leaf extract were slightly lighter in pupal weight, delayed in preimaginal development and showed trends for lower survival	Pilcher <i>et al</i>	Study 11 Page 17
Insect predators on transgenic <i>Bacillus thuringiensis</i> corn	Pollen from Bt-176 maize varieties.	Field study and laboratory study	No acute detrimental effects of the transgenic pollen were observed on preimaginal development, field abundance and survival	Pilcher <i>et al</i>	Study 12 Page 18
Monarch butterfly larvae	Pollen derived from Bt-11 maize dusted on milkweed leaves	Laboratory study	Higher mortality rate in larvae fed with milkweed leaves coated with Bt-pollen	Losey <i>et al</i>	Study 1 Page 19
Monarch butterfly larvae, first instar	Pollen fallen on the leaves of milkweed plants placed inside fields of Bt-176 maize	Laboratory study	Higher mortality rate in larvae fed with leaves coated with Bt-pollen	Hansen/ Obrycki	Study 2 Page 21
Monarch butterfly larvae	Pollen exposure under field conditions	Field studies	Ongoing studies	Expert panel of university and independent researchers**	Page 22

*Studies sponsored by Novartis

**Studies sponsored by various companies

Summary table of studies conducted to assess the effect of Bt- Maize on non-target organisms

Organism studied	CryIA(b) protein applied as	Study design	Result	Authors	Page
Green lacewing and other predators	Whole transgenic Bt-176 maize plant	2 year field study, additional laboratory study	No effect	Pilcher <i>et al</i>	Study 1 Page 23
Various insects including green lacewing	Whole transgenic maize plant	Field study	No effect	Lozzia <i>et al</i> *	Study 2 Page 23
Aphids (<i>Rhodopalosiphum padi</i>) and green lacewings (<i>Chrysoperla carnea</i>)	Whole transgenic Bt-176 maize plant	2 year field study	No effect on aphids or lacewings reared on them	Lozzia <i>et al</i>	Study 8 Page 16 Study 4 Page 24
Green lacewing larvae	ECB larvae having been fed on transgenic or non-transgenic Bt-176 maize varieties	Laboratory study	Green lacewing mortality higher in group reared on Bt-maize	Hilbeck <i>et al</i>	Study 5 Page 24
Green lacewing larvae	Purified and trypsinised Bt-toxin in a specially designed carrier substance	Laboratory study	Increased mortality in larvae fed with Bt-toxin	Hilbeck <i>et al</i>	Study 6 Page 26
Rodents	CryIA(b) enriched Bt-176 maize leaf protein preparations and an appropriate control preparation, or the native bacterial CryIA(b)	Laboratory study	No toxicity was observed	Stillmeadow Inc.*	Study 1 Page 26
Birds (<i>Bobwhite quail</i>)	CryIA(b) protein from enriched leaf preparation from Bt-176 maize and corresponding isogenic control preparations	Laboratory study	No toxicity was observed	Wildlife International Ltd.*	Study 2 Page 27
Broiler chickens	A 38 day feeding study with standard broiler diets prepared with transgenic event 176-derived Bt-corn	Field study	No statistically significant effects in survival or body mass	Brake <i>et al</i> *	Study 3 Page 27

*Studies sponsored by Novartis

2. Introduction

Selective mode of action of CryIA(b) protein

Since 1993, in a continuing effort to monitor the effect of Bt maize on the environment, Novartis has studied the impact of Bt maize on insect populations, among other parameters. Several studies were conducted to assess the environmental safety of the maize containing the CryIA(b) protein. Novartis has sponsored or conducted a number of studies on the effects of Bt maize on non-target insects, including beneficial insects as well as on birds, earthworms, aquatic organisms and mammals.

The overwhelming body of scientific evidence supports the view that non-target species (including beneficial insects) are unaffected by consumption of or exposure to CryIA(b) protein.

The CryIA(b) protein in Novartis Seeds' maize plants is selective in its insecticidal activity; it is only effective against a narrow range of insects within the order *Lepidoptera* (caterpillars), such as the European corn borer, the Southwestern corn borer and *Sesamia*.

The insect species are susceptible to the protein due to the presence of unique binding sites in their guts that 'recognise' CryIA(b) and allow it to exert its toxicity. By eating the maize plant, the corn borer larva takes up the Bt protein in its digestive tract, where it binds to the lining of the corn borer's gut. This leads to holes in the intestinal lining, which is followed by paralysis of the gut. Ultimately the corn borer stops feeding and starves. Only a few moth larvae other than the ECB larvae have the unique binding sites that recognise the CryIA(b) protein. Therefore, the Bt protein is expected not to be toxic to other organisms, which will digest the Bt protein in the same way they digest other proteins. This conclusion is supported by decades of research and widespread field use of Bt-based microbial products.

Mode of action of Bt-sprays

Bt sprays are generally considered to be safe for beneficial insects. They have been used in biological pest control by organic growers and home gardeners for 40 years. However, spores (and in older sprays, β -exotoxins), which are found only in Bt sprays (i.e. not in Bt-plants which only contain the CryIA(b) protein), can contribute to adverse effects on beneficial insects.

Scientific background

Novartis has developed two different lines of genetically modified maize plants:

a) Bt 176

Bt-176 maize was developed by the former CIBA Seeds AG. This maize contains three new genes, *cryIA(b)* from *Bacillus thuringiensis*, *bar* from *Streptomyces hygroscopicus*, and *bla*, the gene for bacterial resistance to ampicillin. The genetically modified maize plants contain two new proteins, a truncated CryIA(b) protein and the *bar* gene product, phosphinothricin-acetyl-transferase (PAT). CryIA(b) is an endotoxin with highly specific insecticidal effects only in lepidopterans and protects the maize plants against corn borer larvae. PAT leads to tolerance against the herbicide glufosinate. Although a bacterial beta-lactamase (*bla*) gene for bacterial resistance to the antibiotic ampicillin is also present in Bt-176 maize, it is not functional in the plants, does not produce a new protein, and cannot confer antibiotic resistance on the plants or organisms that consume the plants.

b) Bt 11

Bt-11 maize was developed by Northrup King, of the former Sandoz Inc. It contains two new genes, *cryIA(b)* from *Bacillus thuringiensis* var. *kurstaki* and *pat* from *Streptomyces viridochromogenes*. The genetically modified maize plants contain two new proteins, a truncated form of CryIA(b) protein and the *pat* gene product, the phosphinothricin-acetyl-transferase (PAT). CryIA(b) protects the maize plants against corn borer larvae, PAT leads to tolerance against the herbicide glufosinate. (see section a) Bt-176 above)

In contrast to Bt-176 maize, there is no bacterial beta-lactamase (*bla*) gene for bacterial resistance to the antibiotic ampicillin present in Bt-11 maize.

The gene product of the *bar* or *pat* gene, PAT, can inactivate phosphinothricin, also referred to as glufosinate ammonium. This substance is a glutamine synthetase inhibitor that has been developed by Hoechst Inc. as a broad spectrum herbicide. Exposure to phosphinothricin causes accumulation of ammonia to cytotoxic levels in all plant cells. The presence of the herbicide tolerance gene facilitates tracking of transgenic plants in breeding.

The genetically modified maize is morphologically indistinguishable from non modified maize. Molecular tools are available to identify all parts of the modified maize plants. The CryIA(b) protein in the genetically modified maize is only active against specific insects. All parts of the plants are safe when fed to animals, or used as base for human nutrition in any form. No hazards relating to health consideration could be identified in the genetically modified maize. For example, extensive side-by-side analyses of the composition of Bt maize grain and conventional grain have shown that they are nutritionally equivalent.

Difference of Bt protein expression in different Bt hybrids

Novartis Seeds developed and sells two lines of genetically enhanced Bt maize hybrids. Those hybrids sold as NK[®] Brand Bt maize with YieldGard[®] insect protection (Bt-11) express the Bt protein in all critical areas of the maize plant. In contrast, hybrids sold as NK[®] Brand Bt maize with KnockOut[®] insect protection (Bt-176) primarily express the Bt protein in the plant's green tissue and pollen.

3. Effects of Bt-Maize on Non-target Insects

(i.e., other than corn borers and other lepidopteran pests of maize)

3.1. Studies on the impact of Bt maize to non-target insects – regulatory and non-regulatory required studies

Novartis has sponsored or conducted a number of studies on the effects of Bt maize on non-target insects, including beneficial insects as well as on birds, earthworms, aquatic organisms and mammals. In fact since 1993, Novartis has conducted field evaluations of insects in maize fields. These ongoing studies, which include research by independent third parties, monitor the effect of Bt maize on non-target insects.

- Field studies comparing the populations of non-lepidopteran insects in plots of Bt maize and non-Bt maize, as well as the impact of a conventional chemical insecticide commonly used on maize. Results of the Bt maize studies indicated no difference in the number of total insects or the numbers in each of the specific groups, such as Coleoptera (e.g., lady beetles), Homoptera (e.g., aphids) and Hymenoptera (e.g., bees). In contrast, treatment with the conventional insecticide had dramatic effects on the total numbers of insects and on the numbers within specific groups, including beneficial insects, compared to the untreated plots.
- Other studies evaluated the effect of Bt maize pollen on lady beetles. No differences in survival or development were observed between lady beetles reared on Bt maize pollen and those reared on non-Bt maize pollen.
- Still more research evaluated the effect of Bt maize pollen on larval honeybees and found the pollen had no effect on larval honeybee development.

These studies found no evidence that exposure to Bt maize protein expressed in maize pollen resulted in any toxic effect on the organism tested. To the contrary, due to its selective toxicity to Lepidopteran pests (e.g., European corn borers), Bt maize will help preserve populations of beneficial insects that might otherwise be threatened by use of chemical insecticides.

Design of the Novartis Seeds regulatory and monitoring studies

The toxicity of the Bt maize CryIA(b) protein to non-target organisms was examined. Two primary test materials were used in these studies: (1) a CryIA(b)-enriched leaf protein preparation (referred to as *Bt* maize protein), obtained by extracting *Bt* maize leaves, enriching the protein for the CryIA(b) fraction, and lyophilising the material to yield a fine protein powder, and (2) pollen collected from *Bt* maize plants (referred to as *Bt* pollen) that were homozygous for the transgenes. In addition, for certain tests comparing the activity of *Bt* maize protein and native CryIA(b), a cell paste containing the CryIA(b) crystal protein produced by fermentation of *Bacillus thuringiensis* var. *kurstaki* strain HD1-9 was used. This was referred to as native CryIA(b). The specific material selected for a study was based on the most likely route of exposure for the organism being tested (e.g., aquatic organisms were exposed to pollen because that is the most likely part of a maize plant expressing CryIA(b) to enter an aquatic environment).

In addition to testing for potential effects of the transgenic plant products against non-target organisms by comparison to negative control groups, non-transgenic maize controls were also used in most of the ecological effects studies. These controls consisted of the same test material (i.e., pollen or maize protein) produced by isogenic (non-transformed) maize plants grown under the same environmental conditions as the transformed maize. By including these controls, effects of the test substance *per se* could be distinguished from effects attributed to the presence of CryIA(b) in the test materials from transformed maize.

Study 1: 48-hour static renewal toxicity of pollen from the genetically modified maize to water fleas (*Daphnia magna*)

Study design

Springborn Laboratories, Inc. conducted a 48-hour static-renewal test with pollen from the genetically modified maize (homozygous for the truncated *cryIA(b)* gene) and isogenic pollen on *Daphnia magna*. Daphnids were <24 hours old at the time of study initiation. For the definitive test, dose levels of 19, 32, 54, 90, and 150 mg pollen/L (containing 5.87 µg CryIA(b)/g pollen) were employed. In addition, isogenic controls at the same pollen concentrations as the treatment group were tested along with a negative control group. Each test or control concentration consisted of two replicates of 10 daphnids each for a total of 20 daphnids/ concentration or control group. Daphnids were exposed for 48 hours with complete renewal of the test solutions after 24 hours.

Results of the study

Mean survival was 100 percent for each of the genetically modified, isogenic, and negative control groups. All daphnids in the genetically modified, isogenic, and negative control groups appeared normal during the study. No immobilisation or sublethal signs of toxicity were observed. The only effect noted was a decrease in dissolved oxygen in the higher test concentrations of both pollen groups. Dissolved oxygen concentrations were inversely related to the concentration of pollen tested and were similar in equivalent concentrations of the genetically modified and isogenic groups. The decrease in dissolved oxygen had no effect on the survival of the daphnids. Higher concentrations for both types of pollen were cloudy and some daphnids were observed to be coated with pollen. At 48 hours, the EC₅₀ based on immobilization was >150 mg pollen/L for both the genetically modified and isogenic groups. Based on these results, the NOEC was 150 mg genetically modified or isogenic pollen/L (the highest concentration tested).

Study 2: Single dose test evaluating toxicity to earthworms (*Eisenia foetida*) using CryIA(b) enriched maize leaf protein

Study design

Springborn Laboratories, Inc. completed a 14-day study on the toxicity of the genetically modified maize to earthworms based on OECD earthworm testing guidelines. Test groups were exposed to the protein preparation from leaves of the genetically modified maize [0.07% CryIA(b)], isogenic protein, or represented a negative control group. A preliminary 14-day study was conducted at 455, 90.9, and 18.2 mg total leaf protein/kg soil. There were no adverse effects on growth or survival of worms at any concentration in the pilot. Based on these results, a single high concentration of 500 mg maize protein/kg soil (0.35 mg CryIA(b) protein/kg soil) was selected for the definitive study. An isogenic control at 500 mg maize protein/kg soil was used in addition to a negative control. Each test or control group consisted of four replicates containing ten worms per replicate (40 worms/concentration). Observations for mortality, toxicity, and behaviour were made on day 7 and day 14. Earthworm body weight was recorded on days 0 and 14. A reference test using chloroacetamide was also used to verify the health of the earthworm culture and the proper sensitivity of the test design.

Results of the study

The LC₅₀ for genetically modified maize protein was determined to be >500 mg protein/kg soil (>0.35 mg CryIA(b) protein/kg soil) and the NOEC was 500 mg protein/kg soil (0.35 mg CryIA(b)/kg soil).

Conclusions of the investigators

No effects on survival or signs of toxicity were noted in the worms exposed to protein preparations of the genetically modified maize, isogenic protein preparations, or in the negative controls on the day 7 observations. After 14 days, no mortality or signs of toxicity were noted in either maize protein group or the negative control. No adverse effects on earthworm body weight after exposure to transgenic protein as compared to worms exposed to isogenic protein or the negative controls occurred. Results of concurrent testing with chloroacetamide verified satisfactory performance of the test design.

Study 3: Effect of pollen from the genetically modified maize on lady beetle (*Coleomegilla maculata*) larval development

Study design

A study assessing the toxicity of *Bt* maize pollen (homozygous for the *cryIA(b)* gene) to lady beetle larval development was conducted at Iowa State University (Ames, IA), according to EPA Guideline No. 154A-23. *C. maculata* larvae were reared on either transgenic maize pollen, isogenic (control) pollen, or an optimal diet of pea aphids (*Acyrtosiphon pisum*). No other food source was available to the lady beetles during the study. Testing was initiated with first instar larvae and these were followed until adult emergence of survivors. Prior to the initiation of the definitive study, a pilot study using hybrid *Bt* maize pollen, which was hemizygous for the transgenes, was conducted. In this pilot study, five out of five *C. maculata* larvae successfully completed development to adults. In the definitive study there were three replicates of 15 larvae/replicate in each test or control group. Larval survival, development times of the different life stages, and adult weight were measured.

Results of the study

Results of the definitive study indicated that survival of *C. maculata* larvae reared on pea aphids was 91%, survival of larvae raised on isogenic control pollen was 43%, and survival of larvae raised on transgenic pollen was 47%.

Conclusions of the investigators

Pea aphids are an insect prey species considered to be an optimal diet for *C. maculata* development and the good survival observed for this group indicates that appropriate environmental conditions for larval development were maintained during the test. The pattern of mortality observed in the two pollen groups was similar, with most mortalities occurring in the later developmental stages (fourth instar through eclosing adult life stages). The observed mortalities were not attributed to the presence of *CryIA(b)* in the transgenic pollen group because of similar effects in the isogenic group; rather it is suspected that the pollen (transgenic and isogenic) may not have provided sufficient nutritional factors for optimal development. Development time in both pollen diet groups was significantly longer in first through fourth instars compared to development time in the pea aphid diet group. There was no significant difference in development time between the two pollen groups. There was no significant difference among the three treatments in pupal development times. The mean weight of emerged adults in the isogenic pollen diet group was significantly less than the mean weight of emerged adults in the transgenic pollen and pea aphid diet groups. There was no significant difference in the weight of adults raised on transgenic pollen compared to those raised on pea aphids.

Due to the high mortality in the isogenic and genetically modified pollen groups, Novartis Seeds is currently developing an improved testing protocol for this study. The low survival of larvae in both pollen groups complicates interpretation of the results; however, several results of this study suggest that the genetically modified nature of the pollen does not affect *C. maculata* larval survival and development. These results include the similar survival patterns and similar developmental times for larvae raised on isogenic and genetically

modified pollen, and the absence of weight differences between surviving adults in the genetically modified pollen group compared to the pea aphid control group. The good survival of the group raised on pea aphids (>90%) indicates that satisfactory environmental conditions were maintained for larval development. The results suggest that a nutritional deficit in maize pollen (both isogenic and genetically modified) may relate to the lower survival of *C. maculata* larvae reared to adult stage on these pollens.

Study 4: Effect of pollen from the genetically modified maize on lady beetle (*Coleomegilla maculata*) larval development

Study design

Pilcher *et al* published the results of a repeat lady beetle study using Bt-176 pollen in a similar protocol, wherein higher survival was obtained by supplementing the diet with pea aphids.

Results of the study

No statistically significant differences in survival or development time were observed between the groups reared on Bt-176 pollen and control pollen, and satisfactory overall survival of pollen (+aphid supplement) groups was observed. Average survival among the lady beetles reared on Bt-176 pollen (supplemented with aphids) was 89%, compared with 69% survival on control pollen (supplemented with aphids) and 64% survival on aphids alone.

Pilcher, C.D.; Obrycki, J.J; Rice, M.E.; Lewis, L.C. **Preimaginal development, survival and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn.** Environm. Entomol. 26 (2), 446-454 (1997).

Study 5: Effect of Bt maize pollen on larval honeybee (*Apis mellifera* L.) development

Study design

Pollen produced by event 176-derived maize plants contains CryIA(b) protein, and ingestion of this genetically modified pollen is anticipated to be the primary route by which honeybees will be exposed to Bt maize. A study conducted by California Agricultural Research (Kerman, CA), evaluated whether ingestion of Bt maize pollen from event 176-derived maize plants had any measurable effects on larval honeybees (*Apis mellifera* L.) maturing within honeycomb brood cells.

A single dose study was conducted in which approximately 1 mg of *Bt* maize pollen and a drop of water was administered as sole source of food of three to five day old honeybee larvae. For comparative purposes, one control treatment included larval bees that received no treatment while a second control group was administered a 1 mg dose of non-transgenic pollen and a drop of water. A positive control involved the incorporation of the carbaryl insecticide Sevin[®] and non-transgenic pollen into the larvae's diet. Each treatment included four replicates of 25 larvae each. Following treatment, the bees were allowed to be capped inside their source hives and later moved to a growth chamber. Those bees surviving to emergence were counted and all treatments were statistically compared to ascertain treatment effects on larval bee survival as well as time to adult emergence.

Results of the study

The studies have been performed twice. Three statistically significant groups were identified: (1) larval bees administered the genetically modified pollen had an average emergence

frequency of 95% (92.5% in the second study), while the untreated group's value was 96% (95% in the second study), (2) a 65% (92.5% in the second study) emergence frequency from the group receiving non-transgenic pollen, and (3) 4% emergence from the carbaryl treated group (second Study: 6.25% from the potassium arsenate treated group).

Conclusions of the investigators

The cause of the reduced emergence frequency in the non-transgenic pollen group is unclear. Relative differences in hive vigour or genetic variability may have contributed. There were no differences observed among any of the groups in the average number of days to emergence, nor were any behavioural effects observed.

Based upon these results, there are no measurable detrimental effects of ingestion of CryIA(b)-containing pollen on larval honeybee development.

To address the reduced emergence in the control pollen group in this study, a second similar study was conducted, at the request of the Canadian authorities. In this study higher amounts of pollen were used (2 mg per cell), a different positive control compound was used (potassium arsenate) and, of most relevance, the treatment groups were represented equally among all the hives, circumventing the problem that arose in the first study. The average survival in this study was 93% among larvae that received control pollen, 95% among larvae that received Bt maize pollen, 95% among untreated larvae, and 6 % among larvae exposed to potassium arsenate (a known insect gut toxin).

Study 6: Effect of Bt maize pollen on larval honeybee (*Apis mellifera* L.) development

Study design

Novartis is currently evaluating potential effects of Bt maize on honeybees under semi-field conditions in the south of Germany near Pforzheim. Mortality, foraging activity and brood development of small colonies originating from the same breeding line is examined. The bee colonies are held in cages placed over plots of Bt-maize and an isogenic non-Bt-maize at the time of pollination of the maize plants. Bees are fed with an auxiliary food source and "api candy" ad libitum.

Results of the study

The study is currently ongoing.

Monsanto did studies on larval and adult honeybees that showed no effect when feeding purified CryIA(b) protein.

Study 7: 28-day survival and reproduction study in collembola (*Folsomia candida*, common name springtails) using CryIA(b)-enriched maize leaf protein

Study design

A 28-day chronic toxicity and reproduction study was conducted by Springborn Laboratories, Inc., using the collembolan, *Folsomia candida*. Collembolans were exposed to one of three soil concentrations of protein extracted from *Bt* maize (corn) leaves ("*Bt* maize leaf protein"), protein extracted from nontransgenic maize leaves ("control maize leaf protein"), or untreated soil. Each test or control group consisted of forty 10-12 day-old collembola subdivided into four replicates of 10 animals each. The concentrations of *Bt* maize leaf protein used were 125 mg protein/kg soil, 250 mg protein/kg/soil, and 500 mg protein/kg soil. The CryIA(b) delta-endotoxin content of the *Bt* maize leaf protein was 0.07%, therefore the test concentrations in terms of CryIA(b) were 0.088, 0.175, and 0.35 mg/kg soil. A concentration of 500 mg control maize leaf protein/kg soil was used as a control for possible effects from maize protein *per se*. The collembolans in each replicate of each treatment group were

provided with 2 mg yeast as food on days 0 and 14. Since collembolans live within soil substrate, it was not possible to observe the condition of the animals until study termination without unduly disrupting the test system. Adult survival and reproduction were analysed at study termination.

Results of the study

The NOEC (no effect concentration) value was found to be 125 ppm, the maximum acceptable toxicant concentration (MATC) range being 125 mg protein/kg soil [0.088 ppm CryIA(b)] to 250 mg protein/kg soil [0.175 ppm CryIA(b)], with a point estimate (geometric mean of the NOEC and LOEC) of 180 mg protein/kg soil [0.126 ppm CryIA(b)].

Points to be considered

Collembolans prefer to feed on saprophytic fungi found on decaying plant matter, rather than on living plants per se (Klironomos, J.N. *et al*, (1992) soil. Biol. Biochem. 24: 685-692.). the scenario of pre-harvest soil incorporation of genetically modified maize is most unlikely. Therefore, collembolans would not be likely to ingest significant quantities of fresh maize plant tissue but rather ingest partially digested plant tissue that has been colonised by fungi. It is likely that the fungi would have degraded some of the CryIA(b) protein present in the plant tissue. Therefore the actual safety margins will likely be greater since the data for the above values assume that the organisms will be exposed to 100 percent of the CryIA(b) protein per hectare of Bt-maize.

Monsanto Company did a collembola study using a different exposure system. Instead of adding the Bt maize leaf protein to artificial soil, they provided it to the organisms on their breeding substrate, a 9:1 (wt:wt) mixture of plaster of Paris and neutralised activated charcoal. The results showed no toxicity to Collembola.

Sims, S.R. and Martin, J.W.. **Effect of the *Bacillus thuringiensis* insecticidal proteins CryIA(b), CryIA(c), CryIIA and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola).** EPA Guidelines, Subdivision M, Microbial and Biochemical pest control, Registrant submitting data. January 18, 1996.

In a second study, conducted by Ricerca Inc. on behalf of Monsanto, collembola were fed a mixture of up to 50% lyophilised material of Bt-maize plants mixed with yeast for 28 days. The results showed no toxicity to Collembola.

Halliday, W.R. **Chronic Exposure of *Folsomia candida* to corn tissue expressing CryIA(b) protein.** Monsanto study number XX-97-064, Monsanto Company, St. Louis, MO 63167. (1997).

Study 8: Impact of transgenic maize expressing truncated CryIA(b) protein on several non-target insect populations: Diptera, Hymenoptera, and Coleoptera (Coccinellid family) as well as Homopterans

Study design

Ciba Seeds conducted a small plot field study in Bloomington, IL during the summer of 1993 to evaluate the impact of maize expressing the CryIA(b) endotoxin on associated populations of insects. The study focused on beneficial predators and parasites in the orders Diptera, Hymenoptera, and Coleoptera (Coccinellid family) as well as Homopterans, which represent an important food source for beneficial predators. Insect populations in transgenic hybrid maize plots were compared to populations in isogenic hybrid maize and wild type maize plots. The study also evaluated the impact of a conventional chemical insecticide, permethrin, on insect populations in maize. There were three replicate plots of each type of

treatment. Insect populations were monitored weekly over a 10 week period from mid-June through early September using Scentry Multigard[®] yellow sticky traps. Two traps were placed in each plot (plots were approximately 7 m long by 3 m wide). Traps were coded at collection and sent to an independent laboratory (Ricerca, Inc) for scoring.

Results of the study

Results of the monitoring study indicated no difference in the number of total insects or the numbers in specific Orders between the transgenic maize plots and either the isogenic or wild type control maize plots. There was no shift in the taxonomic distribution of insects associated with the *Bt* maize compared to the control maize. In contrast, treatment with permethrin had significant effects on the total numbers of insects and on the numbers within specific groups compared to the untreated plots.

Conclusions of the investigators

The beneficial lady beetle predators (coccinellids) were particularly susceptible to permethrin. Coccinellids, dipterans, and hymenopterans represent the majority of beneficial predators and parasites associated with maize. The results of this monitoring study suggest that expression of CryIA(b) in maize should not adversely effect insects in these groups.

Study 9: Effects of CryIA(b) protein on several insect populations: Diptera, Hymenoptera, Coleoptera and Lepidoptera

Study design

The study was carried out in Italy, in the Po valley, in summer 1994, with a different maize hybrid carrying the same genetic modification. There were three replicate plots at two locations for both the genetically modified hybrid and the isogenic control hybrid, which were monitored four times.

Results of the study

Insects belonging to the orders Coleoptera, Diptera, non-target Lepidoptera and Hymenoptera were observed during the whole season in all plots. No significant differences were observed between the plots with the genetically modified maize hybrid and the plots with the non modified maize hybrid. Only the aphid *Rhopalosiphum maidis*, present up to the end of July, was no longer detected later. This disappearance is most likely due to the presence of aphid predators such as Coccinellids and was observed on both the genetically modified maize and the non modified control maize.

Conclusions of the investigators

The results of this monitoring study suggest that expression of CryIA(b) in maize does not affect insects in these groups, except for the expected impact of CryIA(b) protein on the target organisms.

Study 10: Effect of Bt maize on the development of aphids (*Rhopalosiphum padi*) and of green lacewings (*Chrysoperla carnea*), their natural predators

Study design

In a two years study the influences of Bt maize on the development of aphids (*Rhopalosiphum padi*) and of green lacewings (*Chrysoperla carnea*), their natural predators fed on them, was reported. Two different experiments were conducted to separate the impact of insecticidal plants on sucking insects from the prey – predators interaction. The specimens of *R. padi* L. used for the experiment were field collected in spring and

subsequently bred separately in isolation on Bt maize Event 176 or on a nontransgenic hybrid isogenic to it. Stage-specific development times until maturity were recorded. Neonate aphids were placed in isolated cells on the maize plant leaves in the laboratory. The dates after which the exuviate were found were reported, as well as the date of the first birth. To assess fecundity and longevity of *R. padi* viviparous females were isolated on parts of leaves and the number of births or dead viviparous females was recorded daily.

Mortality of *C. carnea* exclusively fed on *R. padi* who had fed upon transgenic and non-transgenic maize was recorded. In daily observations the times relative to pupation and emergence of the adult lacewings were recorded, as were the deaths that occurred before completion of development.

Results of the study

No detrimental effects of transgenic Bt maize on postembryonic developmental time, fecundity or survival of *R. padi* were recorded. Moreover, no influence on preimaginal development or mortality of *C. carnea* were observed when reared on *R. padi* that had fed on Bt maize.

Lozzia, G.C.; Furlanis, C.; Manachini, B. and Rigamonti, I.E.; **Effects of Bt corn on *Rhodopalosiphum padi* (Rhynchota Aphididae) and on its predator *Chrysoperla carnea* Stephen (Neuroptera Chrysopidae)**. Boll. Zool. Agr. Bachic. Ser II, 30 (2): 153-164.

Study 11: Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact of natural enemy populations in transgenic versus isogenic corn

Study design

In a 1994 field experiment, oviposition, predation and parasitism of the European corn borer were recorded in transgenic and isogenic maize plants.

Results of the study

No adverse impact could be detected in the transgenic plants with respect to egg mass predation, parasitism of egg masses and sloughing of eggs of the European corn borer. There was no difference in densities of predators of the European corn borer throughout the oviposition period. Parasitism of European corn borer larvae by *Eriborus terebrans* and *Macrocentus grandii* was not significantly different in transgenic and non-transgenic plots.

Orr, D.B. and Landis, D.L., **Oviposition of European Corn Borer (Lepidoptera: Pyralidae) and Impact of Natural Enemy Populations in Transgenic versus Isogenic Corn** J. Econ. Entomol. 90(4): 905-909 (1997)

Study 12: Effects of Bt maize on non-target arthropods under field conditions

Study design

In a field trial with normal-sized agricultural maize plots in the Burgundy (France) the arthropod fauna (including Lycosidae, Linyphiidae, Opiliones, Staphylinidae, Carabidae, Cicadellidae, Thysanoptera, Anthocoridae, Nabidae, Coccinellidae, Chrysopidae and Chalcoidea) in Bt-176 maize and an isogenic non-transgenic control field was compared with fields, where Bt-sprays or synthetic insecticides were used. Soil-living animals were caught in traps, arthropods on the leaves were counted directly, or the number was determined with knock-off assays. Flying arthropods were caught in so-called yellow traps and counted.

Parasitism of the European corn borer larvae was determined throughout the growing season of the maize plants.

Results of the study

Bt-maize efficiently protects itself against European corn borer.

Neither reduction in quantity, nor any changes in population development could be detected in the soil and plant dwelling fauna. The analysis of the flying fauna has to be completed.

Candolfi, M. et al, **manuscript in preparation.**

Study 13: Field and laboratory evaluations of transgenic *Bacillus thuringiensis* maize on secondary lepidopteran pests (Lepidoptera: Noctuidae)

Study design

Field maize, genetically engineered to produce a protein derived from *Bacillus thuringiensis kurstaki* strain HD-1, was evaluated for its effects on lepidopteran larvae of the noctuid species *Agrotis ipsilon* (Hufnagel), *Papaipema nebris* (Geunee), *Pseudaletia unipuncta* (Haworth), and *Helicoverpa zea* (Boddie).

Results of the study

No effects were observed on larval survival, pupal weight, or days of adult emergence for *A. ipsilon* and *P. nebris*; however, *Pseudaletia unipuncta* (Haworth) reared on *Bt* maize leaf extract were 0.068 lighter in pupal weight, delayed in preimaginal development (from 31 to 41 days) and showed trends for lower survival (11. to 25%), as could be expected for target organisms of the CryIA(b) protein.

Pilcher, C.D.; Rice, M.E.; Obrycki, J.J; Lewis. L.C. **Field and laboratory evaluations of transgenic *Bacillus thuringiensis* corn on secondary lepidopteran pests (Lepidoptera: Noctuidae)** J Econ. Entomol; 90 (2), 669-678 (1997).

Study 14: Preimaginal development, survival and field abundance of insect predators on transgenic *Bacillus thuringiensis* maize.

Study design

Laboratory studies determined the effects of feeding transgenic maize pollen to three different predatory species: 13-spotted lady beetle (*Coleomegilla maculata* DeGeer; Coleoptera: Coccinellidae), insidious flower bug (*Orius insidiosus* Say; Heteroptera: Anthocoridae), and green lacewings (*Chrysoperla carnea* Stephens; (Neuroptera: Chrysopidae).

Results of the study

No acute detrimental effects of the transgenic pollen were observed on preimaginal development and survival.

Pilcher, C.D.; Obrycki, J.J; Rice, M.E.; Lewis. L.C. **Preimaginal development, survival and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn.** Environm. Entomol. 26 (2), 446-454 (1997).

3.2. Studies on the impact of Bt maize to the non-target insects Monarch butterfly and green lacewing

3.2.1. Effect of Bt-maize on Monarch butterflies

The monarch butterfly (*Danaus plexippus*) is an abundant insect. It is one of the most recognised butterflies found from central Mexico to Southern Canada. The monarch is totally dependent on the common milkweed for feeding during the larval stage. Southern Ontario and the northern United States is the traditional summer breeding ground for the monarch while winter migration takes them to the Sierra Chincua mountains near Mexico City, Mexico, to overwinter.

Declining Monarch butterfly populations have been a concern for decades. It is known that many factors play a role in these declines. The most critical is the loss of vital over-wintering habitat in the butterflies' southern winter ranges. Other factors include the effect of insecticides on non-target insects, weed management practices that affect their exclusive milkweed host (mowing of highway right-of ways, ditches and pastures, which destroy the milkweed), urban sprawl, which destroy the habitat where the milkweed will grow, automobile-related mortality and, conceivably, the use of topical Bt sprays.

General remarks on Monarch and the potential effects of Bt maize

A particular variety of maize sheds pollen only during a 5-10 day period. Even within a small geographic area, different maize hybrids will shed pollen at different times due to genetic differences as well as agronomic and environmental factors. It is not known whether female Monarchs would actually chose to lay eggs upon milkweed plants in the presence of maize pollen under field conditions, where pollen-free plants in other locations are available. For many areas of the US, the peak time when larvae will be actively feeding on milkweed plants will occur prior to the peak times of maize pollen shed. For example, the first generation of Monarchs produced in the midwest occurs in June, whereas maize in this area typically sheds pollen during mid-July to mid-August. Moreover, the location of milkweed is outside the range of most pollen drift, since the Iowa State study found that pollen density decreases by 70 percent at the edge of a cornfield, and by 90 percent three meters away from the edge of the cornfield. For these reasons it is likely that the vast majority of monarch larvae throughout their range over a growing season are never exposed to maize pollen in nature at all.

Study 1: The Losey study

Dr. J. Losey *et al.* of Cornell University completed a preliminary laboratory feeding study to assess the potential for Bt maize pollen to affect Monarch butterfly larvae.

Losey, J., L. Rayor and M. Carter. **Transgenic pollen harms monarch larvae.** Nature, 399 (6733), p 214, (1999)

Study design

In a laboratory setting, Monarch butterfly larvae were placed on milkweed leaves artificially coated with pollen derived from Bt maize and non-Bt maize along with leaves free of pollen. Milkweed consumption, larval survival and final larval weight were recorded over a four-day period. The study used pollen from a Bt-11-derived maize hybrid.

Results of the study

Losey *et al.* found a higher mortality rate in larvae fed milkweed leaves coated with Bt maize pollen as compared to larvae fed leaves coated with non-Bt maize pollen and leaves free of pollen.

Conclusions of the investigators

Losey reported that *"larvae of the Monarch butterfly reared on milkweed leaves dusted with pollen from Bt corn ate less, grew more slowly and suffered higher mortality than larvae reared on leaves dusted with untransformed corn pollen or leaves without pollen."* The principle investigator acknowledges that the findings are preliminary. *"Our study was conducted in the laboratory and, while it raises an important issue, it would be inappropriate to draw any conclusions about the risk to monarch populations in the field based solely on these initial results."* (News Release, Biotechnology Industry Organization, June 21, 1999) Indeed, Losey goes so far as to say *"Bt-corn and other transgenic crops have a huge potential for reducing pesticide use and increasing yields. This study is just the first step, we need to do more research and then objectively weigh the risks versus the benefits of this new technology."* (Professor John E. Losey, Cornell Assistant Professor of Entomology, News Release, Cornell University News Service, 19 May, 1999).

Points to be considered

The Losey study discounts that Bt pollen could affect milkweed's palatability, resulting in reduced consumption and/or starvation among the Bt pollen-exposed larvae. Indeed, Losey's data suggests that milkweed without pollen is a preferred food source for Monarch larvae.

The results of the study conducted by Dr. Losey need to be put into perspective. Among our concerns:

- Palatability. The results could indicate the Monarch larvae find milkweed coated in maize pollen unpalatable and thus starvation and/or malnutrition caused the increase in mortality. The author acknowledges this fact saying, *"the reduced rates of larval feeding on pollen-dusted leaves may represent a gustatory response of this highly specific herbivore to the presence of a 'non-host' stimulus."*
- Avoidance. Given this preference, in the wild, where Monarchs encounter milkweed plants both with and without maize pollen, females may avoid laying eggs on milkweed plants laden with pollen.
- Dose-Response. Contrary to common scientific protocol, no attempt was made in this study to establish a dose-response relationship. This means we cannot correlate the amount of Bt pollen the larvae consumed with the degree of larvae mortality observed. Indeed, there was no confirmation that the Bt protein was detectable in the plant pollen.
- Pollen Differences. The study did not consider differences in pollen sources caused by environmental conditions or genetic background. For example, pollen from different maize hybrids is known to vary in moisture level, susceptibility to fungal contamination and amount of natural plant defence compounds. As a result, differences in pollen sources may have contributed to the observed effects. Additionally, following collection, the pollen samples were stored refrigerated in paper bags (instead of the standard storage at -20 to -80°C) for an unspecified period of time.
- Negligible Effect. For many areas of the U.S., June is the peak time when Monarch larvae actively feed on milkweed plants. In contrast, the peak time of maize pollen shed is typically mid-July through early August. Therefore, any potential effect would have a negligible influence on Monarch populations.
- Milkweed Population and Pollen Spread Patterns. Milkweed is not typically prevalent in cornfields, because broadleaf herbicides effectively control the weed. As pollen concentrations are highest in areas immediately adjacent to cornfields, the farther the plant grows from a cornfield, the lower the incidence of pollen. Further, a cornfield typically sheds

pollen only during a five to 10 day period. Given these factors, any potential adverse effects would be confined to those few larvae feeding on milkweed growing in very close proximity to the Bt maize during or immediately following the five-to-10 day window when the cornfield pollinates.

Study 2: The Hansen/ Obrycki study

Dr. L. Hansen and Dr. J. Obrycki of Iowa State University completed a similar preliminary laboratory feeding study to assess the potential for Bt maize pollen to affect Monarch butterfly larvae.

Hansen, L. and Obrycki, J. (1999) "**Non-Target Effects of Bt Corn Pollen on the Monarch Butterfly (Lepidoptera: Danaidae)**," 54th Compiled Proceedings, Annual Meeting, North Central Branch of the Entomological Society of America, abstract.

Study design

Researchers collected milkweed leaf samples from within a Bt maize field and at the edge of the field. Then in a laboratory setting, Monarch butterfly larvae were placed on leaves to assess effects. The study used pollen from maize hybrids derived from Bt-176.

Results of the Study

Hansen/Obrycki found that when first instar Monarch larvae were placed on the collected milkweed samples in the laboratory, 19 percent mortality occurred within 48 hours on leaves containing Bt pollen, as compared to 0 percent mortality on leaves containing non-Bt pollen and 3 percent mortality among larvae not exposed to pollen.

Also of note, the researchers reported finding only low levels of maize pollen on milkweed plants three meters from the cornfield.

Conclusions of the investigators

The study suggests that exposure to Bt pollen may be associated with lower survival in Monarch larvae. The experiment represents a single, unreplicated laboratory trial intended as a preliminary study. The authors intend to do further experiments.

Points to be considered

- No attempts were made to quantify or verify the presence of the CryIA(b) protein in the Bt pollen source, or to confirm the identity or bioactivity of the pollen samples, e.g. by immunoassay or by bioassay against a known CryIA(b)-sensitive lepidopteran pest such as the ECB larvae.
- Contrary to common scientific protocol, no attempt was made in this study to establish a dose-response relationship. This means we cannot correlate the amount of Bt pollen the larvae consumed with the degree of larvae mortality observed. Indeed, there was no confirmation that the Bt protein was detectable in the plant pollen. No data were provided to indicate that equivalent amounts of Bt – and non-Bt pollen were deposited on the milkweed plants representing each pollen-exposure condition.
- The study did not consider differences in pollen sources caused by environmental conditions or genetic background. For example, pollen from different maize hybrids is known to vary in moisture level, susceptibility to fungal contamination and amount of natural plant defence compounds. As a result, differences in pollen sources may have contributed to the observed effects.

- The study cannot distinguish between the possibility that the Bt pollen may have caused or contributed to a true toxic effect, or whether the presence of Bt pollen affected the palatability of the milkweed or had any anti-feedant properties, possibly resulting in avoidance behaviour, reduced milkweed consumption and/or starvation of among the Bt-pollen exposed larvae.
- No attempt was made to use pollen from a non-Bt hybrid that was an isogenic control for the Bt hybrid, and no information is provided to indicate whether the Bt and non-Bt plants were grown under comparable environmental conditions, or whether the pollen was shed or collected under comparable conditions. Different maize hybrids are known to vary in the timing of pollen shed, moisture levels, susceptibility to fungal contamination and in the levels of natural plant defence compounds. Fungal contamination is known to be a limiting factor in the conduct of laboratory insect bioassays with maize tissue (Novartis Seeds data)

Future studies on monarch butterflies

The major companies selling Bt-maize technology today, including AgrEvo USA, Dow AgroSciencesLLC, Monsanto Company, Novartis Seeds, Inc. and Pioneer Hi-Bred International, Inc., have joined together with the American crop Protection Association and the Biotechnology Industry Organisation to sponsor the following important research objectives:

- Distribution of monarch larvae on milkweeds in agricultural and non-agricultural areas
- Pollen deposition and monarch distribution in milkweeds in and around corn fields
- Behaviour of monarch larvae when exposed to Bt- and non-Bt-pollen in choice and no-choice settings
- Overlap of monarch phenology and corn anthesis
- Development of a Bt pollen risk model with sensitivity analysis for key variables, and Assessment of the relative risk of monarch exposure to Bt pollen in the context of the full range of risk factors that impact monarch populations.

3.2.2. Effects of Bt-maize on green lacewing

Lacewing larvae (common green lacewing, scientifically known as *Chrysoperla carnea* and related species) feed on a large number of soft bodied pests, mites and insect eggs. With hollow tusks, called mandibles, they use to pierce it's prey and suck out body fluids. After 14 to 21 days the larvae pupates into a cocoon for about 14 days to emerge as an adult.

Adult lacewings are green with long translucent wings and large golden eyes. Adults feed on nectar, pollen and honeydew to stimulate their reproductive process. An adult female will lay about 200 eggs.

After a few days the eggs hatch and a tiny predatory larva emerges ready to eat the pests.

Lacewing larvae are also known as aphid lions.

Several studies, including laboratory studies, have not detected harmful effects on beneficial insects.

Study 1: Study on various insect predators on Bt maize

Study design

In a two-year field study, combined with an additional laboratory feeding study, beneficial insects, including green lacewings, were exposed to Bt maize pollen and non-Bt maize pollen.

Results of the study

Pilcher *et al* of the Iowa State University concluded that no detrimental effects were observed in the abundance of, among others, the common green lacewing in Bt maize compared with non-Bt maize. In addition, no difference in mortality was found.

Pilcher, C.D.; Obrycki, J.J.; Rice, M.E.; and Lewis, L.C., **Preimaginal Development, Survival, and Field Abundance of Insect Predators on Transgenic *Bacillus thuringiensis* Corn** Environ. Entomol. 26(2): 446-454 (1997)

Study 2: Effects of Bt maize on various arthropods, including green lacewings

Study design

Several field studies were conducted at the Università degli Studi in Milan.

Results of the study

The results indicated no difference in the number or type of insects, which included green lacewings, between the transgenic maize plots and the control maize plots.

Lozzia, G.C and Rigamonti, I.E.; **Prime Osservazioni Sull'Artropodofauna Presente in Campi di Mais Transgenico** ATTI Giornate Fitopatologiche, 223-228; (1998), and

Lozzia, G.C.; Rigamonti, I.E.; and Agosti, M.; **Metodi di Valutazione degli Effetti di Mais Transgenico sugli Artropodi Non Bersaglio** Notiziario sulla Protezione delle piante, 8: 27-39 (1998)

Study 3: Effects of Bt maize on the aphid *Rhodopalosiphum padi* and of the green lacewing *Chrysoperla carnea* feeding on it

Study design

In a two-year study the influences of Bt maize on the development of aphids (*Rhodopalosiphum padi*) and of green lacewings (*Chrysoperla carnea*), their natural predators fed on them, was reported. Two different experiments were conducted to separate the impact of insecticidal plants on sucking insects from the prey – predators interaction.

Results of the study

No detrimental effects of transgenic Bt maize on postembryonic developmental time, fecundity or survival of *R. padi* were recorded. Moreover, no influence on preimaginal development or mortality of *C. carnea* were observed when reared on *R. padi* that had fed on Bt maize.

This study is also described in section ‘Effects of Bt maize on non-target organisms: Study 8, page 16)

Lozzia, G.C.; Furlanis, C.; Manachini, B. and Rigamonti, I.E.; **Effects of Bt corn on *Rhodopalosiphum padi* (Rhynchota Aphididae) and on its predator *Chrysoperla carnea* Stephen (Neuroptera Chrysopidae).** Boll. Zool. Agr. Bachic. Ser II, 30 (2): 153-164 (1998).

Study 4: Effect of the Bt protein (CryIA(b)) on green lacewing (Laboratory feeding study “Hilbeck *et al.*” No.1)

Hilbeck *et al* have completed a laboratory feeding study to assess the potential for genetically modified corn (Bt maize) to indirectly affect a non-target insect species. The purpose of the study was to analyse the effect of the Bt protein (CryIA(b)) on a non-target insect when it eats corn borers, the target of the Bt insecticidal properties.

Hilbeck, A.; Baumgartner, M. Fried, P.M.; Bigler, F; **Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae).** Environm. Entomol. Vol. 27 (2): 480-487 (1998).

Study design

Green lacewing larvae (*C. carnea*), a natural predator of the European corn borer (ECB), were provided with ECB larvae that had fed on either a Bt or a non-Bt hybrid maize plant. In a second part of the experiment (the “control”), green lacewing larvae were provided with a diet of Egyptian cotton leafworm, that had also fed on either Bt or non-Bt hybrids.

When European corn borers eat Bt maize, they become sick and are, therefore, a poor food source for the green lacewing.

Egyptian cotton leafworms were included as a “control”, based on the assumption that they were not sensitive to the CryIA(b) protein produced by the Bt maize plants and, therefore, those which fed on the Bt plants would not be any sicker than those which fed on non-Bt plants.

Because of the differences in Bt toxin sensitivity between the ECB and the cotton leafworms, the authors hypothesised that any differences in effect on the green lacewing predators could be attributable to the Bt toxin. The study used the Bt protein found in Bt-176 (the former Ciba Bt maize).

Results of the study

Hilbeck *et al* found a higher mortality rate (67%) in lacewings reared on Bt-exposed European corn borer larvae as compared to lacewing larvae reared on non-Bt-exposed larvae (37%).

Conclusions of the investigators

The authors conclude that "this was probably due to a combined effect of Bt-exposure via Bt-fed *O. nubilalis* (ECB) and nutritional deficiency caused by sick larvae of poor nutritional value."

Relevance of the findings of the first „Hilbeck et al.“ study

The relevance of the data from the artificial laboratory conditions of the „Hilbeck et al.“ study to an actual field situation is questionable due to the protocol adopted.

For example, the lacewings in the study were provided with a single primary food source, in some cases, only sick, dying prey larvae. In a natural field situation, green lacewing larvae feed on a variety of insect (mainly aphids) and plant species. They would not rely upon a single food source for their development and would not feed exclusively on either Bt-fed or non-Bt-fed ECB larvae.

Points to be discussed

The relevance of the data from the artificial laboratory conditions of the „Hilbeck et al.“ study to an actual field situation is questionable, as the authors themselves conclude: "No conclusion can be drawn at this point as to how increased mortality and differences in development time detected in laboratory trials might translate in the field." The results could be consistent with lacewings that have been fed only "sick" larvae, that is, starvation, malnutrition and/or reduced palatability of the diet could be the cause of increased mortality. Mortality among the lacewing groups fed non-Bt exposed prey was 39% during the course of the study.

The unaffected "control" part of the trial assumes that Egyptian cotton leafworms are **not** sensitive to the CryIA(b) protein and would, therefore, all remain healthy. However, a recent paper by Müller-Cohn *et al.* describes CryIA(b) protein as having low but measurable insecticidal activity in Egyptian cotton leafworm larvae. Therefore, it appears likely that the control group consisting of Bt-exposed Egyptian cotton leafworm larvae were "sick" larvae and also represented a sub-optimal diet.

No data are provided to compare the amounts of larvae consumed by each group, to assess whether decreased palatability of the exposed species may have been a factor. Since the lacewings were not given an alternative diet, starvation among with lacewings provided only with exposed larvae is a reasonable possibility.

There was no attempt to establish a dose-response relationship; i.e. it was not possible to correlate the amount of Bt-exposed prey consumed with the degree of lacewing mortality observed. It is common scientific practice to establish a correlation between the "dose" and the "response" when making claims that an agent is toxic or harmful. Indeed, there was no confirmation that CryIA(b) protein was detectable in the species exposed to Bt plants.

Müller-Cohn, J.; Chaufaux, J.; Buisson, C.; Gilois, N.; Sanchis, V.; and Lereclus, C.; ***Spodoptera littoralis* (Lepidoptera: Noctuidae) resistance to CryIC and cross-resistance to other *Bacillus thuringiensis* crystal toxins**, *J. Econ. Entomol.* 89(4): 791-797 (1996).

Study 5: Effect of the Bt protein (CryIA(b)) on green lacewing (Laboratory feeding study “Hilbeck *et al.*” No.2)

Study design

A second laboratory feeding study, conducted at the Swiss Federal Research Station for Agronomy found an increased mortality rate in green lacewing larvae fed with purified and trypsinised Bt toxin as compared to lacewing larvae not fed with Bt toxin. Laboratory studies were carried out to determine the effects of CryIA(b) on developmental time and mortality of *Chrysoperla carnea* larvae. The CryIA(b) protein was encapsulated into small paraffine spheres together with a liquid diet. After reaching the second instar, all larvae received this artificial diet with or without CryIA(b) protein.

Hilbeck, A.; Moar, W.J., Pusztai-Carey, M., Filippini, A., Bigler, F; **Toxicity of *Bacillus thuringiensis* CryIA(b) Toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae).** Environm. Entomol. Vol. 27 (5): 1255-1263 (1998).

Results of the study

When reared only on artificial diet containing CryIA(b) protein, total immature mortality was 57% compared to 30% in the untreated control. 29% of the Chrysopid larvae that received the CryIA(b) protein later during their larval development, died, compared to 17% in the respective control group. No differences were detected in developmental time between the two groups.

Conclusions of the investigators

CryIA(b) protein is toxic to *C. carnea* larvae at 100µg/ml diet by using encapsulated artificial diet.

Points to be considered

The relevance of the data from the artificial laboratory conditions of the “Hilbeck *et al.*” study to an actual field situation is questionable due to the protocol adopted.

The lacewings in the study were provided with a single primary food source, a specially designed diet leading to up to 40% higher mortality even in the control group without Bt protein. This was most probably due to the high concentration of protein in the paraffin spheres. Lacewing larvae in both groups required significantly higher developmental times when reared on this artificial diet, regardless of exposure to CryIA(b) protein. In a natural field situation, green lacewing larvae feed on a variety of insect and plant species. They would not rely upon a single food source for their development. Novartis therefore believes that relevance of the data from the narrow laboratory conditions of the second “Hilbeck *et al.*” study to an actual field situation is questionable.

4. Studies on the impact of Bt maize to other animals – regulatory required studies

Study 1: Effect of the genetically modified maize on rodents/small mammals

Study design

An *in vitro* digestibility study, and two acute oral toxicity studies in mice were carried out by Stillmeadow Inc., using either a CryIA(b)-enriched maize leaf protein preparation (0.07% CryIA(b) protein by weight) and an appropriate control preparation, or the native bacterial CryIA(b) protein of *B. thuringiensis* var. *kurstaki* strain HD1-9 (65% CryIA(b) protein by weight).

Results of the studies

There were no significant differences in clinical findings or body weight gain in the two treatment groups. The CryIA(b) protein was quickly degraded in simulated mammalian digestive fluid. A complete discussion on the safety of the CryIA(b) protein for ingestion is given in the part of the dossier dealing with food and feed safety.

Conclusions of the investigators

These results indicate that the CryIA(b) protein as produced in the genetically modified maize will be of no toxicological significance to the mammalian wild fauna.

Study 2: Effect of the genetically modified maize on birds (*Bobwhite quail*)

Study design

Wildlife International Ltd. conducted an acute oral toxicity on birds (*Bobwhite quail*) using a single dose of 1.4 mg/kg CryIA(b) protein from enriched leaf preparation from Bt-maize and corresponding isogenic control preparations on 8-week old *Bobwhite quail*. The dose was determined in a rangefinder study for toxicity. Birds were examined twice daily for 14 days.

Results of the studies

No mortality occurred. There were no remarkable necropsy findings in both groups. No adverse effects on body weight or feed consumption could be observed.

Conclusions of the investigators

The acute oral LD50 was established to be >2000 mg CryIA(b) containing leaf preparations/kg equalling 1.4 mg/kg CryIA(b) protein/kg and the NOEL was 2000mg total protein (1.4 mg CryIA(b) protein).

Study 3: Evaluation of transgenic event 176 Bt-maize in broiler chickens

Study design

A 38 day feeding study evaluated whether standard broiler diets prepared with transgenic event 176-derived Bt-maize had any effects on broiler chickens of each sex.

Results of the study

No statistically significant effects in survival, feed efficiency, or body mass were observed between birds reared on diets prepared from transgenic or isogenic non-transgenic maize.

Brake, J.; Vlachos, D.; **Evaluation of transgenic event 176 Bt-corn in broiler chickens.** Poultry Science 77 (5), 648-653 (1998).

5. Useful websites

Novartis homepage:

[www. Novartis.com](http://www.novartis.com)

Information on Monarch butterflies:

www.ipm.iastate.edu/ipm/icm/1999/6-14-1999/monarchbt
[www. monarchwatch.org](http://www.monarchwatch.org)

General information on genetic engineering:

[www. lifescience.de](http://www.lifescience.de)

Pharma Information service for newsgroups: news@interpharma.ch

6. Further reading

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7. Updating

7.1 Newest studies on monarch butterflies (see also chapter 3.2.1)

7.1.2. Potential impact of pollen from Bt -maize Nov. 15/99 from Douglas Powell

Scientists convened in Chicago on November 2, 1999, to share preliminary results of research conducted this summer on the possible effects of genetically-engineered Bt-maize on the Monarch butterfly.

Bt-maize has proven effective at controlling European corn borer, increasing yields and lowering mycotoxin levels since commercial planting began in 1996 (for summary, see Bhatia, *et al.*, 1999). Bt-maize, manufactured by Novartis AG, Pioneer Hi-Bred International Inc. and Monsanto Co., accounted for more than 25 per cent of the 80 million acres of maize planted in the United States in 1998 (Currie, 1999), and about one-third of the field-maize planted in Ontario in 1999 (AgCare, 1999) .

The Bt toxin used in Bt-maize is active against the Lepidoptera family of moths and butterflies, including the Monarch butterfly. However, when Bt-maize was approved in the U.S. and Canada, regulators and scientists reasoned that the impact of Bt-maize -- or more correctly the pollen from Bt-maize containing active toxin -- on Monarch populations would be minimal, given that milkweed, the desired food of Monarch larvae, is rarely found in maize fields, but in adjacent fields, that the toxin is rapidly inactivated by ultraviolet light and drought conditions, and that non-discriminate spraying for other corn pests may present a significantly higher risk to the Monarch population through chemical drift.

In May 1999, a study by Cornell University researchers published in the journal *Nature* (Losey, *et al.*, 1999) indicated that pollen from Bt-maize could kill Monarch caterpillars in laboratory tests. The authors correctly recognized that the study was limited in applicability, and that field tests would be required to determine the significance of this finding in an artificial environment. Upon publication, Dr. John Losey was quoted as saying, "We can't forget that Bt-maize and other transgenic crops have a huge potential for reducing pesticide use and increasing yields. This study is just the first step, we need to do more research and then objectively weigh the risks versus the benefits of this new technology."

Shelton and Roush (1999) responded that although the recent short correspondence in *Nature* reporting a laboratory study in which pollen from Bt-transgenic maize was fed to Monarch butterflies (Losey, *et al.*, 1999) has attracted considerable coverage in the popular press, it has also widespread rebuttals and criticisms in the scientific press (Beringer, 1999; Fumento, 1999; Hodgson, 1999). Shelton and Roush (1999) also state that a previous and more relevant and realistic field study (Hansen and Obrycki, 1999) has been largely overlooked, whereby the authors examined Bt-maize pollen deposition on milkweed plants within, and adjacent to, field maize and then assayed the leaves with first instar larvae. Pollen levels were highest within the field (where Monarchs are scarce), but even there Monarch mortality was only 16 per cent.

In response to the Cornell report, a consortium of biotechnology and pesticide companies -- the Agricultural Biotechnology Stewardship Working Group (ABSWG) -- funded 17 studies to quantify the risk of Bt-maize to Monarchs (Weiss, 1999; Currie, 1999). The research was conducted during the summer of 1999 at universities in maize-producing regions of North America (BIO, 1999). Data presented at the meeting indicated that not all strains of Bt-maize are equally toxic (Brower and Zalucki, 1999); some varieties of Bt-maize may, in a theoretical or laboratory setting, harm the butterfly, while other types may not (Currie, 1999). Furthermore, it was suggested that the amount of pollen migrating to milkweeds was "likely to be dangerous to only those Monarchs feeding on milkweeds within or close to the edges of the maizefields" (Brower and Zalucki, 1999).

Although researchers have much to learn about the ecological consequences of Bt-maize on Monarchs, the findings of the meeting were, according to media accounts and discussions with some participants, generally positive. Stuart Weiss, a Stanford University expert in ecological modeling, was quoted as saying, "the worst-case scenario of this toxic cloud of pollen saturating the maize belt is clearly not the case."

Mark Sears, chair of the department of environmental biology at the University of Guelph and chair of the Ontario Corn Borer Coalition, reported that virtually all pollen grains land within 10 yards from the field, 90 per cent of which travel less than five yards (Weiss, 1999). Sears postulated that the risk of the hazard to Monarch larvae is minimal, especially after discovering that at least 500 grains of pollen per square centimeter of milkweed leaf are necessary to sicken caterpillars. After three days of accumulation during pollination season, Sears found this concentration was barely attained on nearby milkweed leaves.

Iowa State University's John Pleasants found that wind direction, rainfall and other factors significantly affect pollen concentrations on

milkweed. Pleasants found that "88 per cent of milkweed within one meter of a maize field would fall below the level where they could hurt the caterpillars and 100 per cent of the milkweed just two meters from a Bt-maize field would be Monarch-safe" (Kendall, 1999). Such findings on pollen dispersion are especially significant when coupled with planting preferences. Powell *et al.* (1999) found that planting the borders of a maize field to non-Bt maize was the second most prevalent implementation of Bt-refugia guidelines among 400 Ontario maize producers who planted Bt-maize in 1999, and the most common practice among those with more than 100 acres of maize .

John Losey, author of the original Cornell study, was cited as saying he believed that Monarchs might avoid milkweed near maize plants (Kendall, 1999), but also indicated during the meeting that it is "too early to be reassured, or more alarmed, based on this data" (Weiss, 1999).

Various accounts have described different conclusions from the November 2, 1999, meeting of the ABSWG, though all agreed that results were preliminary and studies were far from complete (Yoon, 1999). Brower and Zalucki (1999) identified three key areas of the problem. The effects of Bt maize on Monarch butterflies will depend on distribution and abundance of milkweed within and around the edges of maize fields, oviposition on the milkweeds, and temporal coincidence between susceptible Monarch life stages and pollen shedding from the maize crop. Review of data indicated that basic Monarch biology and ecology were poorly understood, and that data from toxicity bioassays were too preliminary to draw any conclusions. Brower and Zalucki (1999) encouraged researchers to conduct field research during the summer of 2000, exposing cohorts of Monarchs to pollen on field plants within maize fields using various Bt-maize strains and non-Bt maize and wild controls. Toxic and chronic effects of Bt also need to be determined. Other participants, quoted in media accounts and e-mail summaries of discussions at the meeting, quantified the risk to Monarchs, based on preliminary data, as extremely remote.

European corn borer, *Ostrinia nubilalis*, is the most damaging insect pest of maize throughout the United States and Canada. Entomologists estimate that losses resulting from ECB damage and control costs exceed \$1 billion each year (Alstad, 1997; Dekalb, 1998; Andow and Hutchison, 1998; Haag, 1999). ECB typically go through two life cycles during the maize growing season, the 2nd generation usually causing the most damage. In 18 tests over the last six years, Iowa State University researchers saw losses of 4 bu/acre or more from 94 percent of the fields they examined due to ECB (Dekalb, 1998). Very conservative estimates place the value of Bt- maize at \$7-10 million annually in improved maize yields in Ontario in 1998, when about 20 percent of the crop was planted to Bt varieties.

ECB damage also causes human health concerns. Maize kernel feeding by ECB often leads to infection by fungi in the genus *Fusarium*, including the fumonisin-producing species (Munkvold *et al.*, 1999). Fumonisin is a class of mycotoxins. Esophageal cancer in humans has been associated with consumption of maize with high concentrations of the fumonisins (Munkvold *et al.*, 1999).

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