July 9, 2002

Reviewed By: Eric B. Lewis and Anthony Q. Armstrong, Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725

EPA Reviewer: Robyn Rose, Biopesticides and Pollution Prevention Division (7511C)

DATA EVALUATION REPORT

STUDY TYPE: Nontarget Insect Testing Tier I, OPPTS 885.4340

TEST MATERIAL: *Bacillus thuringiensis* Cry3Bb Protein and the Genetic Material Necessary for its Production (Plasmid Insert PHI 8999) in Corn

CITATION:

Author:	G. Head
Title:	Research on the Effects of Corn Rootworm Protected Transgenic Corn Events on Nontarget Organisms: Preliminary Results
Study Completion Date:	March 29, 2002 (Report date - studies not yet completed)
Laboratory:	Various
Sponsor:	Monsanto Co., St. Louis, MO
Laboratory Report ID:	00-CR-032E-7
EPA Reg.No:	Not provided
DP Barcode:	D282664
MRID No:	456530-03

CLASSIFICATION:
Supplemental to completing field studies and submitting final reports to the Agency for review.

ACTIVE INGREDIENT:
Bacillus thuringiensis Cry3Bb1 event MON 863

QUALITY ASSURANCE STATEMENT: No Quality Assurance Statement was included. This MRID is a compilation of progress reports from several different investigators.

GLP COMPLIANCE STATEMENT: A signed GLP compliance statement was submitted that stated this research is not GLP compliant. This MRID is a compilation of progress reports from several different investigators.

REVIEW CONCLUSIONS: Based on preliminary data, MON 863 corn may have the potential to impact some nontarget organisms.

SUBMISSION PURPOSE: To provide progress reports for studies to determine the effects of a transgenic corn rootworm control product (event MON 863) on nontarget organisms. MRID 45653003 contains progress reports for eight ongoing trials being conducted in Kansas, Nebraska, Illinois, Virginia, and South Dakota. A trial is also being conducted in New York, but no progress report was included.

MATERIALS, METHODS & RESULTS: All the trials compared the effects of transgenic corn MON 863 with those of a non-transgenic corn, with or without insecticide. In five of the field trials the corn was planted and monitored at regular intervals to determine the abundance of nontarget insects. Aboveground insect populations were monitored visually and/or by the use of sticky traps and pitfall traps; belowground populations were monitored using Tullgren-type extraction of soil cores. A field trial on plant residue decomposition involved burying bags of corn litter and recovering the bags at regular intervals to determine decomposition rates and litter arthropod populations. One laboratory study fed various combinations of MON 863 or non-transgenic pollen to Coccinellid larvae and measured development and mortality. A second laboratory study infested corn seedlings with plant-pathogenic nematodes and monitored the nematode population for 10 weeks. Details of the individual trials are given below, and a summary is provided in Table 1.

Trial 1, Al-Deeb and Wilde: This study evaluated the effect of MON 863 on non-target arthropods. Field trials were conducted during 2001 in Manhattan (one site) and Scandia (two sites), KS, using MON 863 and appropriate isolines, with or without insecticide. Treatments were conducted in small plots (20 rows x 50 ft or 4 rows x 30 ft). Arthropod abundance was determined using visual counts, pitfall traps, and Tullgren funnels. Visual inspections of 20 randomly-selected plants/treatment were conducted weekly for 3-4 weeks during the silking period, and the number of arthropods was recorded.

Results: Only the visual inspection data have been analyzed. Generally, no significant differences among treatments were found for the abundance (number/plant) of the most common predators, including *Orius insidiosus* (Hemiptera:Anthocoridae) adults/nymphs, *Hippodamia convergens* (Coleoptera:Coccinellidae) adults, *Coleomegilla maculata* (Coleoptera:Coccinellidae) adults, *Scymnus* spp. (Coleoptera:Coccinellidae) adults, or *Hippodamia/Coleomegilla* larvae. The study authors note that these results are consistent with those found in 2000. A table of the 2001 data for each of the three sites was provided in the progress report.

Trial 2, Clark *et al.*: The study was conducted in Nebraska in 2001 to determine the impact of MON 863 on non-target insects, specifically Collembola and Carabids. Treatments included MON 863 and appropriate isolines, with or without insecticide. Plots were 60 x 60 ft. The insects were collected using pitfall traps, and visual observations were made on 10 randomly-selected plants. The timing and duration of sampling were not given in the report.

Results: No significant differences were reported for the number of Carabids or collembolans in the MON 863 and isoline plots. No genus or species identification was provided for the collected insects, and no data were provided in the progress report.

Trial 3, Bitzer and Rice: Studies were conducted during 2000 in Monmouth, IL (one site) and Hampton, NE (three sites) to determine the effect of MON 863 on Collembola (Springtails). Treatments included MON 863 and appropriate isolines, with or without insecticide. Plot size was not provided. In Illinois, soil core samples were taken every two weeks from the end of June until early October. Pitfall traps were installed in June and sampled from mid-July until early October. In Nebraska, pitfall traps were installed (no dates were provided).

Results: MON 863 did not significantly affect the total number of subsurface Collembola found in the Illinois soil core samples, based on analysis of 28 species for which there were at least 20 individuals present. The species were not identified in the report, and the data table referred to in the text was not included. Insecticide use did increase the total number of subsurface Collembola. The Illinois pitfall samples showed that MON 863 did not significantly affect the total number of soil surface Collembola. Insecticide significantly increased the number of surface collembolans, as well as the abundance of several other medium-sized surface insect species. The study authors speculated that the increased numbers were due to the soil insecticide reducing the abundance of a primarily surface-active predator, possibly a Carabid beetle. In the Nebraska trial, 36 surface-active Collembola species (not identified in the report) were captured, and the 14 most common species have been examined for treatment effects. Neither the transgenic corn nor the insecticide significantly affected the total number or species abundance of collembolans, with the possible exception of one Hypogastrid. In that instance, the overall treatment differences were not significant, but the contrast between MON 863 and the isoline suggested that the Hypogastrid was significantly more abundant in the isoline plots (the data table cited in the text was not present in the report). Species richness estimates for the Illinois surface samples were determined using the EstimateS 5.0 program, which examines the cumulative incidence and abundance of species as the number of samples is increased. Results indicate that MON 863 did not reduce species diversity, but insecticide did. Several figures, but no tables, of the data were included in the report.

Trial 4, Zaborski: This study was conducted during 2000 in Champaign, IL to determine the effect of MON 863 on the invertebrate community structure in soil and plant residues. Treatments included MON 863 and appropriate isolines, with or without insecticide. Plot size was 20 x 125 ft. Prior to the 2000 harvest, senesced corn leaves and stalks from each corn line were collected, dried, and reduced to 5-cm pieces. Sub-samples (-3 g) were placed in fiberglass mesh (1 mm) bags and 14 bags of each line were buried 5-10 cm in a non-insecticide, non-transgenic corn plot in February 2001. Two bags from each line were unearthed in April, June, July, August, September, and October, 2001. A modified high gradient extractor was used to extract the microarthropods, and the remaining contents of the bags were oven-dried, ground, and ashed. Soil samples to a depth of 15 cm were also collected from the field, oven-dried, and ashed. A similar experiment using wheat straw was also conducted. Additionally, earthworm populations were sampled by removing one meter of corn plants from the center row at two locations in each plot, placing a 0.5 m^2 metal quadrat over the row, and irrigating the quadrat with two 10 L aliquots of 0.25% formalin solution at 10-minute intervals. Earthworms emerging during the 10 minutes following each irrigation were collected.

Results: Over 26,000 microarthropods representing 62 morphotaxa, including Collembola, mites, and eleven other arthropod taxa have been identified in 87 retrieved litter bags (the taxa were not identified in the report). Only a portion of the data has been partially analyzed, and the only results in the report are for mites. Fungus-feeding Prostigmatid mites initially colonized the material, but populations declined as predatory Mesostigmatid mites increased and fungus-feeding Orabatid mites began to colonize the residues. Late in decomposition, the number of Mesostigmatid declined, while the Orabatid mites persisted, and Astigmatid mites increased (specific data were not provided in the report). The preliminary data suggest that the mite community structure may be slightly different on MON 863 residues than on conventional corn residues, as six morphospecies had an increased abundance (+60 to +450%) on the MON 863 residues. Litter bags from MON 863 contained two and one-half times the number of mites found in litter bags from the non-transgenic isoline. No data were presented for the wheat straw or earthworm studies.

Trial 5, Lundgren and Wiedenmann: In a lab study, *Coleomegilla maculata* larvae were fed either aphids, pollen mixtures containing 0, 25, 50, 75, or 100% MON 863 pollen, or no diet at all. The duration of each instar and the pupal weight were then monitored. Other groups of *C. maculata* larvae were fed one of the pollen mixtures or an artificial diet (not specified), and the duration of larval and pupal stages, pupal weight, adult walking speed, adult flip time, adult survival, and adult fecundity were compared. Similar experiments using Carabids are also being conducted.

Results: There were no significant differences in any of the parameters for *C. maculata* larvae among the pollen-fed treatments. All the pollen-fed treatments had faster larval development, increased survival, and higher pupal weight than the aphid or artificial diet treatments. No data were provided in the progress report. Comparable experiments with Carabids have not been completed.

Trial 6, Lewis et al.: In a non-insect lab study included in MRID 45653003, three-week-old MON 863 and control corn seedlings in pots were infested with 5000, 10,000 or 15,000 *Meloidogyne incognita* (a plant-pathogenic nematode) eggs per plant. Two weeks after infestation, 6 plants per treatment were removed and their roots were stained with fuchsin and pressed between glass plates. *M. incognita* juveniles present were then counted with a dissecting microscope. Five weeks after infestation, 12 plants per treatment were removed and nematode eggs were extracted from the roots using sodium hypochlorite and counted under a dissecting microscope. Ten weeks after infestation, 12 plants per treatment were removed and the nematode egg extraction and counting were again performed. In a separate soil leachate experiment, MON 863 and controls were grown in pots for 4 weeks. The seedlings were then removed and the soil was mixed 1:1 (w/w) with water, shaken for two minutes in plastic bottles, passed through a 125 μ sieve, and centrifuged for 10 minutes at 2000 rpm. The supernatants were transferred to Petri dishes and approximately 100 laboratory-reared *Caenorhabditis elegans* (a bacteriovorous nematode) of mixed life stages or *Steinernema carpocapsae* (an entomopathogenic nematode) juveniles were added to each dish. After 24 hours at -25°C, the number of live and dead nematodes was determined. In a separate root extract experiment, MON 863 or control roots were finely chopped, blended at high speed for 5 minutes, and passed through 125 μ and 25 μ sieves. The filtered extract was then used to assess the mortality of *C. elegans* and *S. carpocapsae* using the same method as that used for the soil leachates.

Results: Populations of *M. incognita* juveniles were significantly lower in MON 863 roots than in conventional corn roots at all three inoculation rates. The number of eggs was significantly lower in MON 863 roots after both 5 and 10 weeks (significance was specified in the text, but not shown in the accompanying figure). Twenty-four hour survival of *C. elegans* was 10% in MON 863 extracts, compared to 57% in control extracts, a significant reduction. Soil leachates had no effect on survival, indicating that MON 863 corn might be unlikely to affect nematodes not associated with the corn plant itself. Twenty-four hour survival of *S. carpocapsae* was not affected by root extracts from either MON 863 or control seedlings. The study authors speculated that since the *S. carpocapsae* used in the test were nonfeeding stages, the Bt protein from the root extracts may have to be ingested to be lethal.

Trial 7, Fuller and Mc Manus: The study was conducted in Brookings, SD during 2001. Four plots (1.6-ha) of MON 863 and 4 plots of a traditional hybrid were planted with and without soil insecticides and monitored for Coccinellids by whole-plant counts and sticky traps (16/plot). The whole-plant counts were conducted weekly on 100 randomly-chosen plants and included eggs, larvae, pupa, and adults. Below-ground samples were collected using Tullgren-type extraction of soil cores or hand sorting of soil samples. No additional information was provided in the report.

Results: Coccinellid egg density for the overall growing season was not significantly affected by any of the treatments, although a two-fold increase in egg number was seen in the MON 863 fields during the second week of sampling. The study authors speculated that the increase may have been due to the transgenic plants being more attractive at that time to ovipositing females, although no difference was apparent to the sampling crew. The number of Coccinellid larvae was significantly increased (approximately 2-fold) on the MON 863 plots. However, the increased larval numbers did not result in a greater number of pupae or adults. A separate analysis of *Coleomegilla fuscilabrus*, the most abundant adult Coccinellid in each of the fields, found no significant differences among the treatments.

Trial 8, Ellsbury and French: The study was conducted in Brookings, SD during 2001. Treatments included MON 863 and appropriate isolines, with or without insecticide. Carabid density was measured by placing a single pitfall trap inside each of eight corn earworm emergence enclosures per 4-acre plot. Propylene glycol was used as the collection fluid/preservative in the traps. Weekly counts of emerging adult Carabids were taken, and corn rootworm beetle numbers were counted at the end of the season. The density of Carabids was measured separately using 8 pitfall traps placed between corn rows in June and checked weekly through the harvest.

Results: The cumulative mean number of Carabids was lowest for the MON 863 corn, but no statistical analysis of that data was discussed in the progress report. Analysis of the weekly collection data showed that the number of Carabids was significantly lower in the MON 863 plots compared to controls, but not compared to the insecticide-treated plots. However, the study authors state that the results cannot be regarded as definitive due to missing values resulting from animal damage and other unknown factors. Additionally, there was apparently a density gradient across the plots, and a large wetland between the MON 863 and the other treatments which may have limited the movement of ground-dwelling insects and biased the results. The next set of tests is to include a rearrangement of plots to ensure complete randomization.

REVIEWER'S COMMENTS: All these studies are ongoing, and in most cases only very preliminary results have been included in MRID 45653003. Lack of data, especially tables, in the progress reports makes any assessment of most of the studies cursory. Results from several studies include only the number of insects determined, without identifying the genus/species, which would be meaningful. Five of the eight trials found a significant effect due to MON 863. Bitzer and Rice suggest that MON 863 resulted in a decreased population of one of 14 species of Collembola compared to insecticide treated plots, and preliminary results from Zaborski suggest that the mite community structure may be slightly altered on MON 863 plant residues. A third trial, Ellsbury and French, found a decrease in the number of Carabids in MON 863 plots, but the data are suspect due to plot layout problems. Fuller and McManus reported that the number of Coccinellid larvae, but not pupae or adults, was increased on MON 863 plots, and Lewis *et al.* found that MON 863 plants had lower numbers of *M. incognita* eggs and juveniles. While the progress reports do not include enough data to allow any definitive conclusions, the early information appears to indicate that MON 863 corn may affect some nontarget organisms. Further analysis of the data already collected, as well as additional results from the ongoing trials, are needed before an assessment of the effects of transgenic corn on nontarget organisms can be fully developed.

Table 1. Summary of trials to evaluate the effect of MON 863 on nontarget organisms					
Investigators (Start date)	Location (Study date) Plot size	Treatment	Type of monitoring	Investigator's conclusion	Comments
Al-Deeb and Wilde (2000)	Manhattan and Scandia, KS (2001) Small plots - 20 rows x 50 ft or 4 rows x 30 ft	MON 863 and isolines with or without insecticide	Weekly visual inspection of leaf, silk, and tassel of 20 randomly selected plants during silking	Generally no significant differences in *Orius* nymphs and adults, *Hippodamia*, *Coleomegilla*, *Scymus* adults, or ladybeetle larvae.	Pitfall and Tullgren funnel data not yet analyzed.
Clark et al. (2000)	Nebraska (2001) 60 x 60 ft	MON 863 and isolines with or without insecticide	Visual inspection of 10 randomly selected plants; pitfall traps	No significant difference in carabid or Collembola numbers.	Other arthropods collected, data not analyzed
Bitzer and Rice (2000)	Illinois , Nebraska (2000) not given	MON 863 and isolines, with or without insecticide	Pitfall traps, Tullgren funnels	No apparent effect of MON 863 on Collembola or species diversity. Soil insecticide increased abundance of some species and reduced species diversity.	Soil insecticide apparently increased abundance of some species due to effect on predatory arthropods.
Zaborski (2000)	Illinois (2001) 20 x 125 ft	MON 863 and isolines, with or without insecticide	Mite populations on leaves and stalks from each line buried in litter bags; mite populations in soil; earthworms	Mite community structure slightly different in MON 863 litter	A study comparing wheat straw with corn litter results and a study on earthworm populations are ongoing.
Lundgren and Wiedenmann (2000)	Lab study (2001)	Pollen (0-100% transgenic), aphids, artificial diet, or no diet fed to *Coleomegilla maculata* larvae	Instar duration and pupal weight; duration of larval and pupal stages; walking speed; flip time; survival; and adult fecundity	MON 863 pollen did not affect the measured parameters.	Feeding studies with Carabidae are underway.

Table 1. Summary of trials to evaluate the effect of MON 863 on nontarget organisms

Lewis et al. (2001)	Lab study	MON 863 and isoline seedlings infested with plant pathogenic nematode (*M. incognita*) eggs and monitored for 10 weeks; non-plant pathogenic nematodes (*E. elegans*, S. *carpocapsae*) in soil exposed to MON 863 or isoline root extracts or soil leachates	*M. incognita*: root penetration and egg production; *E. elegans*, S. *carpocapsae*: mortality	*M. incognita*: root population and egg production significantly reduced on MON 863 seedings. *E. elegans*: numbers significantly lower in MON 863 root extracts, no difference in soil leachate. S. *carpocapsae*: No difference in root extract or soil leachate.	S. *carpocapsae* used were non-feeding juveniles.
Fuller and McManus (2001)	South Dakota (2001) 1.6 ha	MON 863 and isolines with and without soil insecticides	Whole-plant counts of coccinellid eggs to adults on 100 random plants/week; sticky traps	No significant differences in egg, pupal, or adult density; 2-fold increase in larval density	
Ellsbury and French (2001)	South Dakota (2001) 407 x 407 ft	MON 863 and isoline with and without soil insecticide	Pitfall traps	Significant differences in number of carabids, likely due to plot layout rather than treatment	
Losey (2001)	New York				Preliminary report not available