UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

NOV 29 2007

MEMORANDUM

SUBJECT: Review of the proposed insect resistance management plan submitted by Monsanto for MON 89034 X MON 88017 Bt corn. EPA Reg. No. 524-LTA. DP Barcode: D335189. Decision: 339469. MRID#: 469513-04, 469513-06, 470794-02, 470794-03.

TO: Susanne Cerrelli, Regulatory Action Leader
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (7511P)

FROM: Alan Reynolds, Entomologist
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (7511P)

Action Requested

BPPD[1] has been asked to review information provided by Monsanto Company on insect resistance management (IRM) for MON 89034 x MON 88017, a new Bt corn product that expresses the Cry1A.105, Cry2Ab2, and Cry3Bb1 toxins. The IRM materials were submitted in several volumes, which included an overall summary of the proposed IRM plan ("Insect Resistance Management Plan for the Combined Trait Product MON 89034 x MON 88017"; MRID# 469513-06). Additional studies covering efficacy against the target pests were also included (MRID#s 469513-04 and 470794-03). A separate volume (MRID# 470794-02) was submitted to address questions previously raised by BPPD in a preliminary screen of the IRM data and is also included in this review.

Conclusions

1) Monsanto has provided information to demonstrate that the dose of MON 89034 x MON 88017 against the major target pests should be comparable to the dose of the MON 89034 and

[1] The use of BPPD in this review refers to the BPPD IRM Team

MON 88017 isolines. Therefore, the IRM considerations (dose, refuge, cross resistance) for MON 89034 and MON 88017 are applicable to the stacked MON 89034 x MON 88017 product.

2) Monsanto has proposed a 5% lepidopteran refuge as part of the "Separate Refuge" option for MON 89034 x MON 88017 corn. Due to uncertainties in the review of the MON 89034 IRM plan (see BPPD 2007a), a 5% refuge cannot be supported at the present time. Instead, BPPD recommends that the separate refuge option include a 20% lepidopteran refuge (as has been required for other Bt corn products). However, BPPD notes that a 20% refuge can be supported for MON 89034 x MON 88017 in cotton-growing regions in southeastern U.S. where a 50% refuge has been previously required.

3) Monsanto's proposal for a combined refuge (covering both coleopteran and lepidopteran pests) is acceptable. This option calls for a 20% refuge throughout the U.S. (as described in #2 above, a 20% refuge can be supported in southern cotton-growing regions).

4) The other aspects of Monsanto's IRM plan for MON 89034 x MON 88017 including resistance monitoring, remedial action plans, grower education, compliance, and annual reporting are acceptable. Resistance monitoring (sampling, bioassays, and data reporting) and remedial action should be conducted under the terms and conditions of registration for MON 89034 and MON 88017.

Background

MON 89034 x MON 88017 expresses the Cry1A.105, Cry2Ab2, and Cry3Bb1 Bt toxins and is targeted against lepidopteran corn pests including European corn borer (ECB), southwestern corn borer (SWCB), corn earworm (CEW), and fall armyworm (FAW) as well as the coleopteran corn rootworm sp. pest complex (CRW). MON 89034 (Cry1A.105 and Cry2Ab2) provides activity against the lepidopteran corn stalk and ear insects while MON 88017 (Cry3Bb1) is active against root-feeding CRW. The product was created by conventional breeding in which the previously-registered MON 88017 (EPA Reg. No. 524-551) was crossed with MON 89034 (EPA Reg. No. 524-LTL). The Cry3Bb1 toxin in MON 88017 is the same as expressed by MON 863 corn (Yieldgard Rootworm, EPA Reg. No. 525-528), which was registered by Monsanto for the 2003 growing season.

An IRM plan for MON 88017 was reviewed and approved when the product was registered in December, 2005. The elements of the plan were largely the same as those implemented for MON 863 corn (registered in 2003) and consisted of the following refuge requirements (taken from BPPD 2005a):

- Growers must plant a structured refuge of at least 20% non-corn rootworm protected Bt corn that may be treated with insecticides as needed to control corn rootworm larvae. Growers will not be permitted to apply CRW labeled insecticides to the refuge for control of insect pests while adult corn rootworms are present unless the Cry3Bb1 field is treated in a similar manner.
- Refuge planting options include: refuge acres should be planted as blocks adjacent to MON 88017 corn fields, perimeter strips, or as in-field strips.
- External refuges must be planted adjacent (e.g., across the road) to Cry3Bb1 MON 88017 fields.

- Refuges planted as strips across the field must be at least 4 rows wide, preferably 6 consecutive rows wide.
- Insecticide treatments for control of corn rootworm larvae may be applied. Instructions to growers will specify that insecticides labeled for control of corn rootworm adults cannot be applied while adults are present in the refuge unless the Cry3Bb1 field is treated in a similar manner.
- If the refuge is planted in a field that is in a crop rotation system, then MON 88017 must also be planted in a field that is in a crop rotation system.
- If the refuge is planted on continuous corn, then the MON 88017 field may be planted on either continuous or in a field that is in a crop rotation system.

Additional requirements for resistance monitoring, remedial action (in the event of resistance), compliance monitoring, grower education, and reporting were required as part of the MON 88017 IRM plan.

IRM considerations for MON 89034 have been reviewed separately (see BPPD 2007a). The conclusions from that review that are germane to the stacked MON 89034 x MON 88017 product will also be discussed in this memorandum.

Given that IRM plans for both MON 88017 and MON 89034 have been previously evaluated, this review will focus on IRM considerations solely for the MON 89034 x MON 88017 stacked product. Included in this assessment will be plant expression and efficacy studies to verify the dose of the MON 89034 x MON 88017 product and a discussion of the refuge strategy for a stacked product simultaneously targeting lepidoptera and coleoptera.

MON 89034 x MON 88017 IRM Plan Considerations

The following sections describe BPPD's review of Monsanto's IRM plan and additional resistance management considerations for MON 89034 x MON 88017. The plan as proposed by Monsanto is summarized in the Data Evaluation Report (DER) for MRID# 469513-06 that is attached to this review.

Dose

Dose, or the amount of toxin expressed by the transgenic crop relative to the susceptibility of the target pests, is a critical component of IRM. Models have shown that a high dose of toxin, coupled with a non-transgenic refuge to provide a supply of susceptible insects, is the most effective strategy for delaying resistance in Bt crops. The 1998 Science Advisory Panel (SAP) defined high dose as a level of toxin 25 times greater than is needed to kill all susceptible insects and outlined five techniques to determine high dose.

The MON 89034 x MON 88017 corn product was created using conventional breeding of the MON 89034 and MON 88017 hybrids. Dose against the target pests has been previously established for both MON 89034 (see BPPD 2007a) and MON 88017 (see BPPD 2005b). MON 88017 (Cry3Bb1) has been characterized as a "low to moderate dose" against CRW. MON 89034, on the other hand, expresses two toxins (Cry1A.105 and Cry2Ab2) and has close to a high dose against the target pests (ECB, SWCB, CEW, and FAW). These dose characterizations are described in the context of the SAP high dose definition, although products with less than a

high dose may still be effective at controlling the target pests while maintaining compatibility with IRM strategies. In the case of a two gene product, the toxins collectively may produce an "effective" high dose. In this case, each toxin by itself may not supply a high dose, but in combination a sufficient control (>95% of heterozygotes) is provided to be considered high dose. MON 89034 may be an example of an effective high dose, although key questions remain regarding the overall dose profile of the product (see the dose discussion in BPPD 2007a).

Since dose has been previously established for the isolines, the dose of the stacked MON 89034 x MON 88017 product can be verified relatively easily. Two confirmatory sets of data are typically analyzed: 1) plant expression data to verify that the toxin expression in the stacked product is at least as high as in the single gene isolines; and 2) efficacy data to confirm that the level of control against the target pests in the stacked product is at least as high as the isolines. Data were provided in Monsanto's submission to address both of these points.

Plant expression data were provided in MRID# 469513-03 and were separately as part of the product characterization assessment of the product (see BPPD 2007b). However, data relevant to the discussion of dose will also be summarized in this review. Monsanto tested a variety of plant tissue samples (leaf, root, pollen, and grain; dry weigh and fresh weight) for all three toxins in the stacked product (Cry1A.105, Cry2Ab2, and Cry3Bb1). For Cry1A.105, the amount of toxin (both dry and fresh weight, on a µg/g basis) in the stacked MON 89034 x MON 88017 exceeded that in MON 89034 for all tissues tested except grain (which was not significantly different). Expression of Cry2Ab2 in the stack was also comparable to the MON 89034 isoline, with no statistical differences between any of the tested tissues. Likewise, Cry3Bb1 toxin levels between MON 89034 x MON 88017 and MON 88017 were similar and not statistically different in all of the tissue types analyzed. Based on these results, it can be concluded that the level of toxin expression in MON 89034 x MON 88017 corn tissues is the same as in the MON 89034 and MON 88017 isolines.

In addition to the toxin expression data, Monsanto conducted a series of efficacy trials with the key lepidopteran and coleopteran target pests. The first set of studies (contained in MRID# 469513-04) was conducted in 2006 and evaluated efficacy against western corn rootworm (WCRW) and fall armyworm (FAW). A second study (MRID# 470794-03) was submitted that focused on the major lepidopteran target pests (including ECB, SWCB, CEW, FAW, sugarcane borer, western bean cutworm, and black cutworm).

A summary of the methodology and results of the first study for FAW and WCRW is described in the Data Evaluation Report for MRID# 469513-04) that is attached to this memorandum. As described in the DER, the studies were conducted with the stacked MON 89034 x MON 88017 corn product as well as MON 89034 and MON 88017 isolines. Trials were conducted in growth chambers which allowed for controlled conditions to evaluate artificial infestations of FAW and WCRW larval feeding. Protection against FAW was determined by assessing leaf feeding damage; the results showed that FAW feeding on MON 89034 x MON 88017 corn was not statistically different than on the MON 89034 isoline (or with a stacked product of MON 89034 crossed with a non-Bt, herbicide tolerant line). Leaf damage was significantly higher on non-Bt expressing hybrids and MON 88017 (which does not express lepidopteran active toxins). For WCRW, efficacy was evaluated with root feeding damage ratings. Both MON 89034 x MON 88017 and the MON 88017 isoline had the lowest nodal injury scores and were not statistically different from each other. Conversely, MON 89034 alone (with no CRW active toxins) and the non-Bt control hybrid had the statistically greater damage than the hybrids with MON 88017.

Based on these results, it can be concluded that the stacked MON 89034 x MON 88017 product will have comparable efficacy as the MON 89034 and MON 88017 isolines for FAW and WCRW. However, this conclusion can not be extended to other lepidopteran target pests (including ECB, SWCB, and CEW) since they were not evaluated in this study.

To evaluate the other lepidopteran target pests of MON 89034 x MON 88017, Monsanto submitted a second volume detailing field efficacy studies that were conducted during 2005. These studies were not included as part of the original registration application, but were submitted in response to a request from EPA (see EPA letter to Monsanto dated January 17, 2007). A full summary of the studies is contained in the DER for MRID# 470794-03 attached to this memorandum. Seven lepidopteran pests were evaluated, including ECB, SWCB, CEW, FAW, sugarcane borer (SCB), western bean cutworm (WBCW), and black cutworm (BCW). Artificial and natural infestations were used and efficacy was determined by assessing feeding damage. For ECB, SWCB, SCB, FAW, and CEW the efficacy of MON 89034 x MON 88017 was comparable to MON 89034. In addition, both MON 89034 and MON 89034 x MON 88017 provided equivalent or superior control than the previously-registered MON 810 against these target pests. Trials for WBCW and BCW did not directly evaluate MON 89034 x MON 88017, although MON 89034 was shown to more efficacious than MON 810, although limited locations were tested. Given the results, MON 89034 x MON 88017 can be expected to provide comparable efficacy as MON 89034 and control a broader range of lepidopteran corn pests than MON 810.

Overall, the protein expression data and efficacy studies demonstrate that MON 89034 x MON 88017 corn has an equivalent dose profile as MON 89034 corn. Therefore, the conclusions derived from the dose assessment of MON 89034 can be applied to the MON 89034 x MON 88017 product. The review of the IRM plan for MON 89034 (see BPPD 2007a) concluded that both Cry1A.105 and Cry2Ab2 are expressed at sufficient levels to provide a high level of control (>90%) against the four major lepidopteran target pests (ECB, SWCB, CEW, and FAW). However, BPPD noted that much of the dose information was circumstantial; MON 89034 was only directly evaluated with leaf disk assays. Other data for dose were obtained from susceptibility assays with purified protein (that were compared to MON 89034 expression data) and tests with (non-commercialized) single gene isolines. In addition, the data do not appear to support a "high dose" as defined by the 1998 SAP (a level of toxin 25 times greater than needed to kill susceptible larvae; i.e. a dose greater than the LC_{99} of the pest). Some survival of MON 89034 plant tissue was noted for ECB, SWCB, and CEW. Monsanto assumes that the survivors would not reach adulthood due to growth inhibition (and therefore are functionally dead), but that assumption was not tested due to the short time frame of the experiment.

To support their IRM proposal for MON 89034, Monsanto has cited a model developed by Dr. Rick Roush (1998) for dual toxin plant-incorporated protectants (PIP). This model assumes that the two toxin PIP exerts at least 95% control of susceptible homozygotes and 70% control of heterozygotes (with one resistance allele). MON 89034 may provide 95% control of susceptible homozygotes (though this was not conclusively demonstrated by the dose data), but the potential mortality of heterozygotes was not addressed. Because of this, BPPD recommended that Monsanto submit additional dose information to support the IRM strategy for MON 89034 (see BPPD 2007a).

Proposed IRM Plan for MON 89034 x MON 88017 Corn

Since MON 89034 x MON 88017 is a stacked product targeting both lepidopteran and coleopteran (corn rootworm) pests, a refuge plan that accounts for both pest complexes must be designed. Such a plan was created for a previously-registered stacked product containing MON 88017 and MON 810 (Cry1Ab). This plan included options for separate refuges (for CRW and lepidoptera) or a combined refuge for both pest complexes. The specific plan is described fully in the EPA fact sheet for MON 88017 x MON 810 (see BPPD 2005a). Monsanto's proposal for MON 89034 x MON 88017 largely follows this previously-developed framework for a stacked lepidopteran/coleopteran product.

The specific elements of Monsanto's proposed refuge plan for MON 89034 x MON 88017 are listed below (a full description can be found in the DER for MRID# 469513-06 attached to this memorandum). Growers of MON 89034 x MON 88017 must plant one of the two following refuge options:

1. Common Refuge Option: Growers are required to plant a minimum of 20% non-Bt corn as a structured refuge (applicable for the entire U.S.). The refuge must be placed within or adjacent to the MON 89034 x MON 88017 field as either continuous blocks (next to the MON 89034 x MON 88017 field), continuous blocks (within the MON 89034 x MON 88017 field), strips around the MON 89034 x MON 88017 field, or strips within the MON 89034 x MON 88017 field. Strip refuges must be at least four rows wide. Refuges and MON 89034 x MON 88017 acres should be managed under comparable agronomic regimes. In MON 89034 x MON 88017 fields where corn is cropped continuously, refuge acres should be planted in a continuous cropping regime. The refuge may be placed on first-year corn acres only if the MON 89034 x MON 88017 corn is planted on first-year corn acres. Growers have the option of applying conventional insecticides to the corn refuge for control of corn rootworm larvae and other soil pests. The refuge can also be treated with a non-Bt insecticide to control late season pests such as corn borer or corn rootworm adults, although if adult corn rootworm are present, then the MON 89034 x MON 88017 acres must be treated in a like manner. Microbial Bt insecticides cannot be applied to the refuge acres.

2. Separate Refuge Option: Under this option, growers plant two distinct refuges for coleopteran (CRW) and lepidopteran target pests. The **CRW Refuge** consists of a minimum 20% non-CRW protected corn (i.e., corn not containing Cry3Bb1 protein) that must be placed within or adjacent to the MON 89034 x MON 88017 field. Refuge deployment options include continuous blocks adjacent to the MON 89034 x MON 88017 field, continuous blocks within the MON 89034 x MON 88017 field, strips around the MON 89034 x MON 88017 field, or strips within the MON 89034 x MON 88017 field (strips must be at least four rows wide). This refuge size applies throughout the US and could be planted with lepidopteran-protected corn (e.g., MON 89034). Refuges and MON 89034 x MON 88017 acres should be managed under comparable agronomic regimes. In MON 89034 x MON 88017 fields where corn is cropped continuously, refuge acres should be planted in a continuous cropping regime. The refuge may be placed on first-year corn acres only if the MON 89034 x MON 88017 corn is planted on first-year corn acres. Conventional insecticides or seed treatments may be applied to the corn refuge for control of corn rootworm larvae and other soil pests. The refuge can also be treated with a non-Bt insecticide to control late season pests, although if adult corn

rootworm are present then the MON 89034 x MON 88017 acres must be treated in a like manner. The **Lepidopteran Refuge** consists of a minimum 5% non-Bt corn structured refuge (for regions in which no cotton is grown -- i.e., the northern corn belt). In areas where cotton is grown, a minimum 20% non-Bt corn structured refuge must be deployed. Lepidopteran refuges must be placed within ½ mile (within ¼ mile preferred) of the MON 89034 x MON 88017 and CRW refuge fields. When planting the refuge in strips, the strips must be at least four rows wide. Conventional insecticides or seed treatments can be used in the refuge for control of corn rootworm larvae. Insecticide treatments for control of European corn borer, southwestern corn borer and corn earworm may be applied only if economic thresholds (as determined by methods recommended by local or regional professionals) are reached for one or more of these target pests. For both the CRW or lepidopteran refuges, microbial Bt insecticides cannot be applied to either the refuge acres.

The major difference between Monsanto's refuge plan for MON 89034 x MON 88017 and the previously developed plan for MON 88017 x MON 810 is that the company has proposed a 5% lepidopteran refuge instead of a 20% refuge. To support a reduction in lepidopteran refuge, Monsanto has cited a model developed by Dr. Rick Roush (1998) for dual toxin products. This model suggests that smaller refuges may be possible for two toxin products, provided that at least 95% of susceptible homozygotes and 70% of heterozygotes are controlled and there is no cross-resistance between the toxins. As described in the dose section above, Monsanto's MON 89034 data (reviewed in BPPD 2007a) do not conclusively demonstrate 95% homozygote and 70% heterozygote control. In addition, BPPD recommended that Monsanto provide further information on the cross resistance potential of Cry1A.105 and Cry1Fa and Cry1Ac (the Cry1A.105 protein is a chimeric protein consisting of Domains I and II and the C-terminus of Cry1Ac and Domain III of Cry1Fa). Because of these shortcomings, BPPD concluded that a 5% lepidopteran refuge was not justified for MON 89034. However, BPPD noted that registration of MON 89034 could proceed with a 20% refuge (the standard that has been used for Bt corn refuge since 2000). If Monsanto is able to sufficiently address the deficiencies noted in BPPD's review of MON 89034, a reduction in the refuge to 5% could be supported. In a related discussion, BPPD did conclude that a 20% refuge for MON 89034 could be supported for regions in which cotton is grown (i.e. southeastern states) -- these regions have historically required a 50% refuge for Bt corn.

Given the conclusions from the MON 89034 IRM review, BPPD cannot at the present time recommend a 5% refuge for MON 89034 x MON 88017 in the "Separate Refuge" option discussed above. Rather, the current 20% refuge for Bt corn should be used until Monsanto is able to address the uncertainties described above and in the MON 89034 IRM review (BPPD 2007a). On the other hand, Monsanto's proposal for a "Combined Refuge" of 20% for MON 89034 x MON 88017 should be viable. This is because BPPD determined that a 20% refuge for MON 89034 could be supported in cotton-growing regions instead of the 50% refuge required for other Bt corn products. The other aspects of refuge deployment described in the Monsanto's combined and separate refuge options for MON 89034 x MON 88017 corn (including proximity restrictions, strip row width, and insecticide treatment) are compatible with the requirements previously developed for other CRW and lepidopteran IRM programs.

Monsanto's IRM plan for MON 89034 x MON 88017 also included discussions of resistance monitoring, remedial action plans, grower education, compliance, and annual reporting. Resistance monitoring (sampling, bioassays, and data reporting) and remedial action plans for

Cry2Ab2, Cry1A.105, and Cry3Bb1 and the major target pests will be conducted under the registrations for MON 89034 and MON 88017. All other aspects of the IRM program for the stacked MON 89034 x MON 88017 product (remedial action plans, grower education, compliance, and annual reporting) should be made to be compatible with the IRM programs for MON 89034 and MON 88017 registrations.

References

BPPD, 2005a. Fact sheet for MON 88017 and MON 88017 x MON 810 corn. Located at: http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_006430-006502.htm

BPPD, 2007a. Technical Review of Monsanto Company's Submissions (dated September 22, 2006 and March 9, 2007) Regarding Insect Resistance Management for MON 89034 Corn (EPA registration 524-LTL). S. Matten and A. Reynolds memorandum to S. Cerrelli, November 6, 2007.

BPPD, 2007b. Review of Product Characterization and Human Health Data for MON 89034. R. Edelstein memorandum to S. Cerrelli, .

Roush, R.T. 1998. Two toxin strategies for management of insecticidal transgenic crops: pyramiding succeed where pesticide mixtures have not? Phil. Trans. R. Soc. Lond. 353:1777-1786.

DATA EVALUATION RECORD

EPA Secondary Reviewer: Alan Reynolds	
STUDY TYPE:	IRM Plan for MON 89034 x MON 88017
MRID NO:	46951306
DP BARCODE:	DP 335189
TEST MATERIAL:	Transgenic corn event MON 89034 x MON 88017
SPONSOR:	Monsanto Company, 800 North Lindbergh Blvd, St. Louis, MO 63167
TESTING FACILITY:	Monsanto Company, 800 North Lindbergh Blvd, St. Louis, MO 63167
TITLE OF REPORT:	Insect Resistance Management for the Combined Trait Product MON 89034 x MON 88017
AUTHOR:	Graham Head
STUDY COMPLETED:	September 15, 2006
CONCLUSION	Monsanto presents data to support a proposed IRM plan for MON 89034 x MON 88017. This proposed IRM plan is based on the EPA-approved IRM plan for YieldGard Plus®. All components of the plan are the same as YieldGard Plus except for a reduction in refuge size for corn borer and the frequency of reporting to EPA on resistance monitoring for corn rootworm. Monsanto proposes a reduction in the refuge size for corn borer (separate refuge option only) from 20% to 5% in the non-cotton growing region and 50% to 20% in the cotton growing region. Monsanto also proposes to report on resistance monitoring for corn rootworm on a biennial basis.
CLASSIFICATION	Acceptable; pending approval of the reduced refuge sizes proposed in the IRM plan for MON 89034 (MRID 465914-30).
CONFIDENTIALITY CLAIMS	None; a signed statement of no claim of confidentiality was provided (p. 2).
GOOD LABORATORY PRACTICE	Non-GLP study; a signed GLP statement was provided (p. 3).

MONSANTO=S PROPOSED IRM PLAN FOR COMBINED TRAIT MON 89034 x MON 88017

A. Introduction

This volume was developed to support a FIFRA Section 3 Registration for Monsanto=s combined trait MON 89034 x MON 88017 corn which produces the *Bacillus thuringiensis* (Bt)-derived insecticidal proteins Cry1A.105, Cry2Ab2 and Cry3Bb1 which are active against lepidopteran pests. MON 89034 x MON 88017 is produced through conventional breeding by crossing corn inbreds containing the individual events MON 89034 and MON 88017. The Bt proteins produced in MON 89034 x MON 88017 are effective in controlling damage caused by the two most economically important pests in corn: corn borers *(Ostrinia nubilalis* and *Diatraea grandiosella)* and corn rootworms *(Diabrotica* spp.).

To minimize the risk of these pests evolving resistance to CrylA.105, Cry2Ab2 and Cry3Bbl, an insect resistance management plan (IRM) for MON 89034 x MON 88017 has been developed. The key elements of this plan are a combination of the approved YieldGard Plus® (registered trademark of Monsanto Technology LLC) plan and the recently submitted MON 89034 plan (MRID 469514-30). The specific requirements of the MON 89034 x MON 88017 plan are described below. Where the MON 89034 and MON 88017 plans differ (e.g., in refuge size in some regions and in the distance allowed between the refuge and Bt field), the MON 89034 x MON 88017 IRM plan employs the most conservative elements from each.

B. General Elements of the IRM Plan

- Refuge Requirements - Planting of a common non-Bt corn refuge or planting of separate CRW and CB refuges.
- Grower Agreements - Growers will be required to sign contractual agreements, which impose an obligation on each grower to comply with the refuge requirements.
- Grower Education - A program to educate growers about IRM requirements for MON 89034 x MON 88017 will be developed and implemented.
- Compliance Assurance Program - A program to evaluate and promote grower compliance with IRM requirements will be developed, implemented, and reported to EPA.
- Monitoring - Programs to evaluate whether there are statistically significant and biologically relevant changes in target insect susceptibility to CrylA.105, Cry2Ab2 or Cry3Bb1 protein will be developed, implemented, and reported to EPA.
- Mitigation - A remedial action plan in the event that any insect resistance is detected will be developed and (if triggered) implemented.
- Reporting to EPA - Annual reports on sales of MON 89034 x MON 88017 seed, IRM grower agreement results, compliance assurance, and educational programs will be submitted on or before January 31 of each year.

The MON 89034 x MON 88017 refuge requirements are described in Section C below. Descriptions of the remaining elements of the MON 89034 x MON 88017 IRM plan are described in Sections D through I and are based on the submitted MON 89034 IRM plan and the approved MON 88017 IRM plan. The IRM plan for MON 88017 was approved by EPA in 2005 (Vaughn, 2001).

C. Refuge Requirements

Growers may choose to plant a common refuge for both CB and CRW pests, or they may choose to plant separate CB and CRW refuges. For growers who do not typically apply insecticides for control of CB, the common refuge option may be appropriate. In regions where CB infestations are high, growers may benefit from planting separate CB and CRW refuges. This option is appropriate when CB infestations are expected to exceed economic thresholds. The benefit of a separate refuge allows the grower to manage CB in both the CRW refuge (by planting CB-protected corn in this refuge) and CB refuge (by applying conventional insecticides) without having to spray the entire MON 89034 x MON 88017 field (see Common Refuge Option: Refuge Management below).

1. Common Refuge Option

a. **Refuge Size:** Growers must plant a minimum of 20% non-Bt corn as a structured refuge. This refuge size applies throughout the US.

b. **Refuge Placement and Configuration:** The refuge must be placed within or adjacent to the MON 89034 x MON 88017 field. Refuge deployment options include continuous blocks adjacent to the MON 89034 x MON 88017 field, continuous blocks within the MON 89034 x MON 88017 field, strips around the MON 89034 x MON 88017 field, or strips within the MON 89034 x MON 88017 field. When planting the refuge in strips, the strips must be at least four rows wide.

c. **Refuge Management:** The refuge and MON 89034 x MON 88017 acres should be managed under comparable agronomic regimes. In MON 89034 x MON 88017 fields where corn is cropped continuously, refuge acres should be planted in a continuous cropping regime. The refuge may be placed on first-year corn acres only if the MON 89034 x MON 88017 corn is planted on first-year corn acres. Growers have the option of applying conventional insecticides to the corn refuge for control of corn rootworm larvae and other soil pests. The refuge can also be treated with a non-Bt insecticide to control late season pests such as corn borer or corn rootworm adults. However, if growers opt to treat the refuge while adult corn rootworm are present, then the MON 89034 x MON 88017 acres must be treated in a like manner. Microbial Bt insecticides cannot be applied to the refuge acres.

2. Separate Refuge Option

a. **The CRW Refuge**:

i. **Refuge Size:** Growers must plant a minimum of 20% non-CRW protected corn (i.e., corn not containing Cry3Bbl protein) as a structured refuge. This refuge size applies throughout the US. This refuge should be planted with CB-protected corn (e.g., MON 89034). A separate CB refuge also must be established (see CB Refuge below).

ii. **Refuge Placement and Configuration:** The refuge must be placed within or adjacent to the MON 89034 x MON 88017 field. Refuge deployment options include continuous blocks adjacent to the MON 89034 x MON 88017 field, continuous blocks within the MON 89034 x MON 88017 field, strips around the MON 89034 x

MON 88017 field, or strips within the MON 89034 x MON 88017 field. When planting the refuge in strips, the strips must be at least four rows wide.

iii. **Refuge Management:** The refuge and MON 89034 x MON 88017 acres should be managed under comparable agronomic regimes. In MON 89034 x MON 88017fields where corn is cropped continuously, refuge acres should be planted in a continuous cropping regime. The refuge may be placed on first-year corn acres only if the MON 89034 x MON 88017 corn is planted on first-year corn acres. Growers have the option of applying conventional insecticides or seed treatments to the corn refuge for control of corn rootworm larvae and other soil pests. The refuge can also be treated with a non-Bt insecticide to control late season pests. However, if growers opt to treat the refuge while adult corn rootworm are present then the MON 89034 x MON 88017 acres must be treated in a like manner. Microbial Bt insecticides cannot be applied to the refuge acres.

b. The CB Refuge:

i. **Refuge Size:** Growers in counties in which no cotton is grown (e.g., counties in the northern corn belt) must plant a minimum of 5% non-Bt corn as a structured refuge. Growers in counties in which cotton is grown must plant a minimum of 20% non-Bt corn as a structured refuge.

ii. **Refuge Placement and Configuration**: The refuge must be placed within ½ mile (within ¼ mile preferred) of the MON 89034 x MON 88017 and CRW refuge fields. When planting the refuge in strips, the strips must be at least four rows wide.

iii. **Refuge Management**: Growers have the option of applying conventional insecticides or seed treatments to the refuge for control of corn rootworm larvae. Insecticide treatments for control of European corn borer, southwestern corn borer and corn earworm may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). Microbial Bt insecticides cannot be applied to the refuge acres.

Table 1 delineates and compares the refuge requirements for MON 89034, MON 88017 and the combined traits in MON 89034 x MON 88017. In this table, only the common refuge regime for MON 89034 x MON 88017 is described. A separate refuge for CB and CRW is allowed and recommended when growers expect to treat for CB in the refuge. Refuge size is the same under all three IRM plans in counties where cotton is grown (i.e., a minimum of 20%) but differs in counties without cotton. Additional differences occur in refuge placement and management. Figures 1 through 4 provide illustrations of the various refuge configurations for MON 89034, MON 88017, and MON 89034 x MON 88017.

TABLE 1. Comparison of refuge requirements in the MON 89034, MON 88017, and MON 89034 x MON 88017 IRM plans.

Requirements	MON 89034	MON 88017	MON 89034 x MON 88017
Refuge Corn Types	Conventional	Conventional MON 89034 (a CB refuge planted ≤½ mile also will be required)	Conventional MON 88017 (a additional refuge for ECB will be required)

	Roundup Ready® Corn	Roundup Ready corn	Roundup Ready corn
Refuge Size	≥ 5% in counties where no cotton is grown; ≥ 20% where cotton is grown	≥ 20%	≥ 20%
Refuge Placement	≤ ½ mile (≤ ¼ mile preferred)	Adjacent or within field	Adjacent or within field
Refuge Configurations	Block, in-field strips (≥ 4 rows), or edges	Block or strips (≥ 4 rows)	Block or strips (≥ 4 rows)
Crop Rotation	No restriction regarding rotation between con and other crops.	If the refuge is planted on rotated ground, then Cry3Bbl corn must also be planted on rotated ground; if the refuge is planted in continuous corn, the Cry3Bbl field may be planted on either continuous or rotated ground.	If the refuge is planted on rotated ground, then Cry3Bbl corn must also be planted on rotated ground; if the refuge is planted in continuous corn, the Cry3Bb1 field may be planted on either continuous or rotated ground.
Insecticide Use	Insecticide treatments can be used in refuge to control ECB/SWCB when above economic thresholds.	Conventional insecticides or seed treatments can be used in refuge to control CRW larvae & other soil pests. If the refuge is treated with a foliar insecticide labeled for CRW control when CRW adults are present, then MON 88017 also must be treated.	Conventional insecticides or seed treatments can be used in refuge to control CRW larvae & other soil pests. If the refuge is treated with a foliar insecticide labeled for CRW control when CRW adults are present, then MON 89034 x MON 88017 also must be treated.
Microbial Insecticides	Microbial Bt insecticides are not allowed.	(Not applicable)	Microbial Bt insecticides are not allowed.

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Table reproduced from pg. 10, MRID 46951306

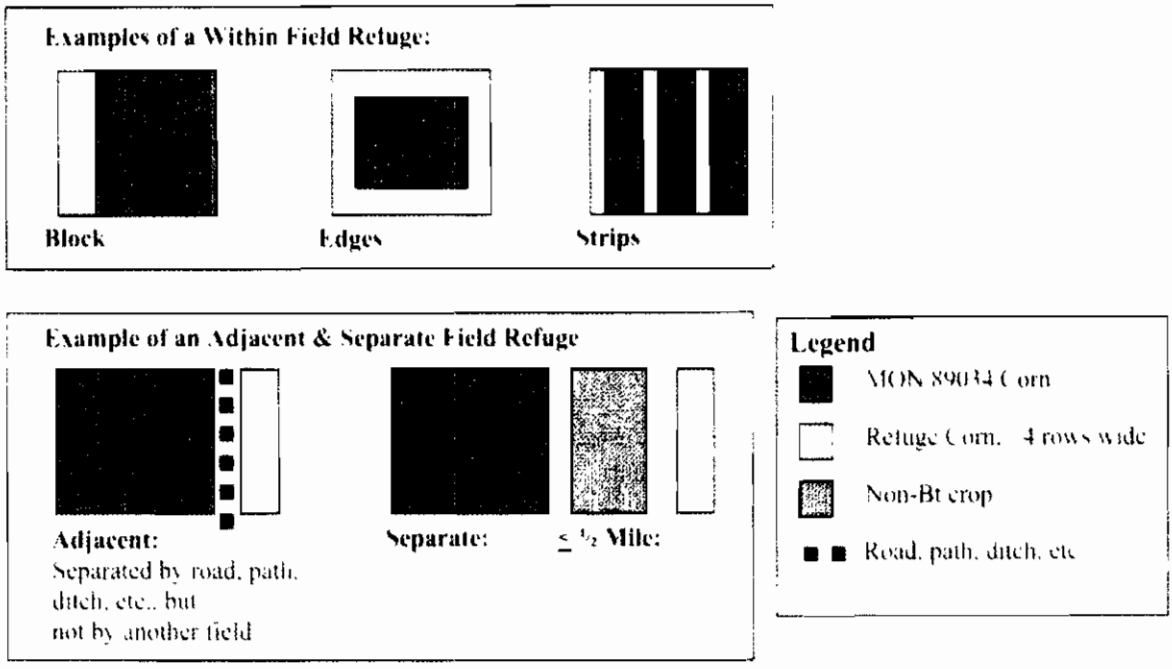


Figure 1. MON 89034 corn refuge placement and configuration options

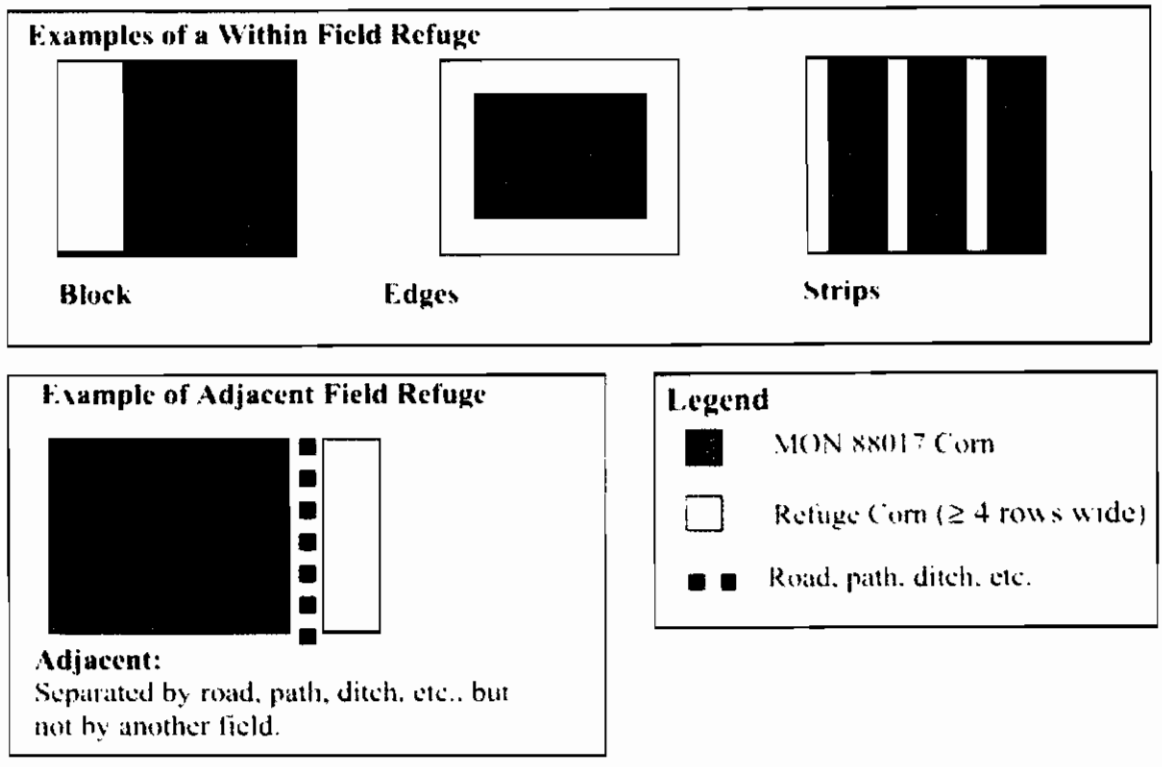


Figure 2. MON 88017 corn refuge placement and configuration options.

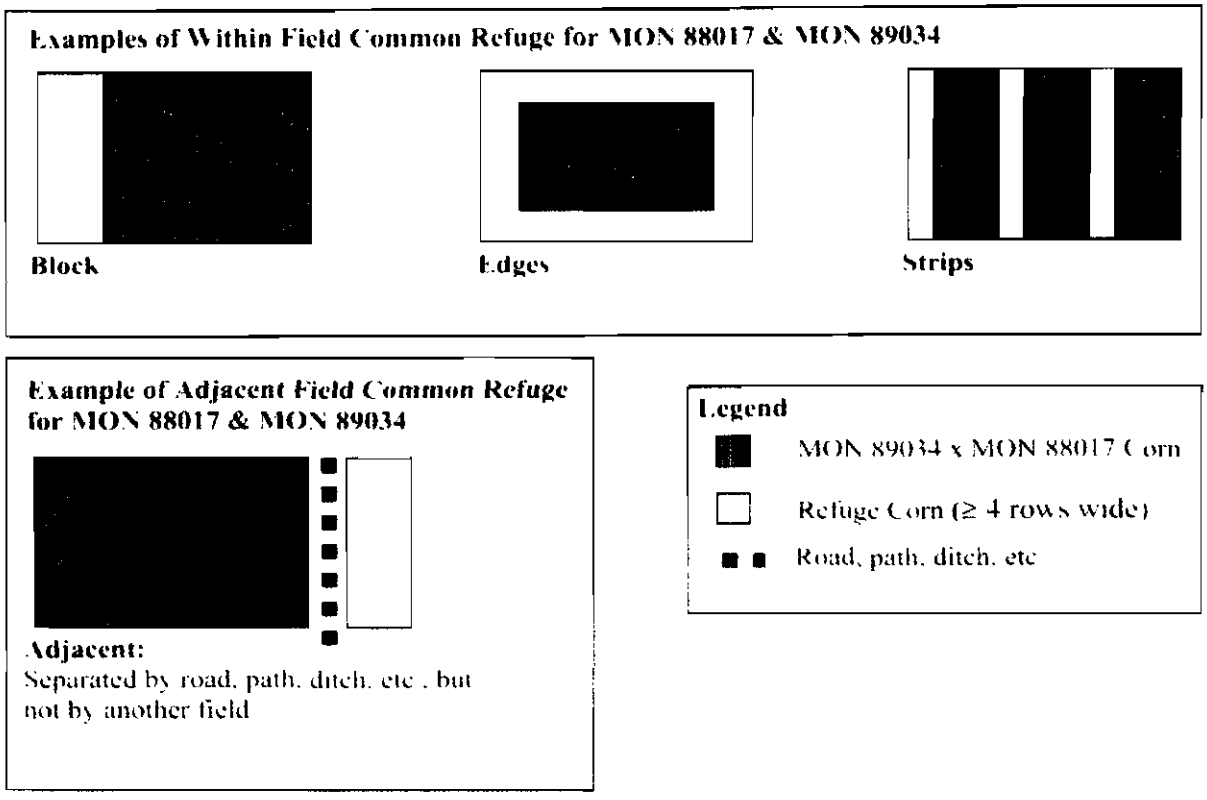


Figure 3. MON 89034 x MON 88017 corn: Common refuge placement and configuration options for MON 88017 & MON 89034.

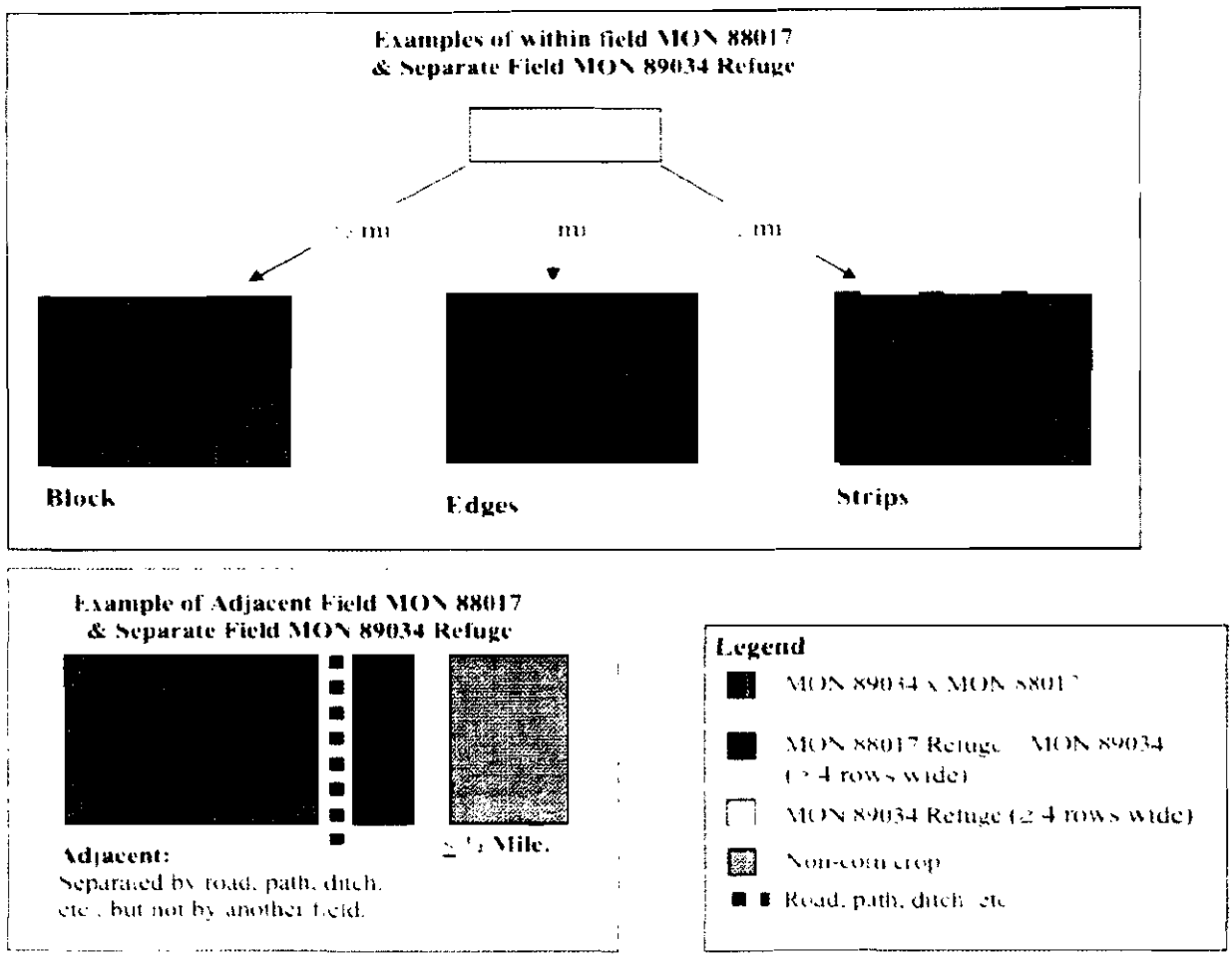


Figure 4. MON 89034 x MON 88017 Separate refuge placement and configuration options for MON 88017 & MON 89034

D. Grower Agreements

Each grower will be required to sign a contractual agreement, which imposes an obligation on each grower to comply with the refuge requirements. There are no differences in this requirement from what is in place for MON 88017 and other Bt corn products.

E. Grower Education

Monsanto will develop and implement programs to educate growers about the MON 89034 x MON 88017 IRM requirements. These programs will be integrated into the programs already in place for MON 88017 and other Bt corn products.

F. Compliance Assurance Plan

Monsanto will develop, implement and report to EPA on programs to evaluate and promote grower compliance with JRM requirements. These programs will be identical to those already in place for MON 88017 and other Bt corn products.

G. Monitoring

Monsanto will develop, implement and report to EPA on programs to evaluate whether there are statistically significant and biologically relevant changes in target insect susceptibility to CrylA.105, Cry2Ab2 or Cry3Bbl protein. The monitoring regimes for the target insects *Ostrinia nubilalis* (ECB), *Diatraea grandiosella* (SWCB), *Helicoverpa zea* (CEW) and *Diabrotica virgfera* (WCR) are described in each of the MON 89034 and MON 88017 IRM plans. The MON 89034 x MON 88017 plan will not change the monitoring programs for these target insects.

H. Mitigation

Monsanto will develop and, if triggered, implement a Remedial Action plan in the event that any insect resistance is detected. Remedial Action Plans for suspected resistance to CB or CRW are described in their respective IRM plans, where the MON 89034 x MON 88017 plan would not change either of these Remedial Action plans. For CB, the Remedial Action Plan is attached as Enclosure 1 in the EPA amendment letter for CrylAb-expressing corn (EPA Registration No. 524-489) dated October 12, 2001. For CRW, the Remedial Action Plan is described in the IRM plan for corn event MON 863 (p. 13, MRID 455770-01).

I. Reporting

Monsanto will submit annual reports on product sales, IRM grower agreement results, compliance, and educational programs on or before January 31st each year. The reporting for MON 89034 x MON 88017 will not differ from what is described in the CrylAb expressing corn amendment letter from EPA (EPA Registration No. 524-489) dated October 12, 2001, except that the insect resistance monitoring results for CRW will be reported every other year, compared to annually for CB. The rationale for this difference in reporting schedules is attributable to life cycle differences between the pests. Corn borers have two generations per year, whereas CRW has only one generation per year.

Table 2 outlines the education, compliance, resistance monitoring, and mitigation requirements for MON 89034, MON 88017 and MON 89034 x MON 88017. These elements of the plan are essentially identical for all three products. The only difference of significance is in resistance monitoring, where CB susceptibility is assessed annually and CRW susceptibility is assessed on a two-year cycle.

TABLE 2. Comparison of the reporting, education, compliance, monitoring and mitigation elements between the MON 89034, MON 88017 and MON 89034 x MON 88107 IRM plans

Requirements	MON 89034[1]	MON 88017	MON 89034 x MON88017
Acreage	Annual MON 89034 sales	Same as MON 89034	Same as MON 89034

Reporting	data.		
Grower Agreements	Use of Grower agreements Annual affirmation of IRM obligation.	Same as MON 89034	Same as MON 89034
Education	Technology Use Guide describing the IRM requirements; bag tags, sales brochures and other company literature	Same as MON 89034	Same as MON 89034
Compliance Assurance Plan	Grower telephone survey System for receiving inquiries and complaints. On Farm Assessments	Same as MON 89034	Same as MON 89034
Monitoring	Annual assessment of susceptibility for ECB, SWCB & CEW.	Susceptibility for WCRW, NCRW & MCRW assessed every two years. Contingent on availability of diagnostic dose assay.	Annual susceptibility for ECB, SWCB & CEW. Susceptibility for WCRW, NCRW & MCRW assessed every two years. Contingent on availability of diagnostic dose assay.
Mitigation and Remedial Action Plan	Confirmation of reported resistance. Notify customer & Extension Agents. Stop sales and distribution in effected area. Recommend alternative control measures. Notify EPA within 30 days of confirmed resistance. Notify EPA within 90 days of mitigation measures and long term IRM plan.	Same as MON 89034	Same as MON 89034

[1] These requirements are the same as for YieldGard® Corn Borer (MON 810).

J. Summary

This proposed IRM plan for MON 89034 x MON 88017 is based on the EPA-approved plan for YieldGard Plus. All components of the plan are the same as YieldGard Plus except for a reduction in refuge size for corn borer and the frequency of reporting to EPA on resistance monitoring for corn rootworm. We propose a reduction in the refuge size for corn borer (separate refuge option only) from 20% to 5% in the non-cotton growing region and 50% to 20% in the cotton growing region. We also propose to report on resistance monitoring for corn rootworm on a biennial basis. The proposed changes in the plan, compared to the plan for YieldGard Plus, are justified in the MON 89034 IRM volume for refuge size and in Section I of this application for reporting frequency of CRW monitoring.

Reviewer's Comments

1) See 2007 IRM Guide from Monsanto for separate refuge options for corn rootworm and corn borer (attached by ORNL). The same proposed reduction in refuge size is requested for MON 89034 x MON 88017 for corn borer in non-cotton growing areas (20% to 5%) and cotton growing areas (50% to 20%) as described in the MON 89034 IRM plan for the separate refuge option only. The acceptability of these proposed refuge reductions is contingent upon Monsanto addressing all of the uncertainties identified for the MON 89034 IRM plan (see BPPD review of MRID# 469514-30 and 470794-02). If the reduced refuge IRM plan for MON 89034 is approved, these reduced refuge sizes will also be valid for MON 89034 x MON 88017.

References

Vaughn, T., D. Ward, J. Pershing, 0. Head and J. McFerson (2001). An Interim Insect Resistance Management Plan for Corn Event MON 863: A Transgenic Corn Rootworm Control Product. MSL17556, a plan prepared by Monsanto Company. MRID 455770-01

DATA EVALUATION RECORD

EPA Secondary Reviewer: Alan Reynolds

STUDY TYPE:	Product Performance (Nonguideline)
MRID NO:	46951304
DP BARCODE:	DP335188
DECISION NO:	371190
SUBMISSION NO:	Not provided
TEST MATERIAL:	MON 89034 x MON 88017
STUDY NO:	06-01-50-02
SPONSOR:	Monsanto Company 800 N. Lindbergh Blvd. St. Louis, MO 63167
TESTING FACILITY:	Monsanto Company Agronomic Traits 700 Chesterfield Parkway West St. Louis, MO 63017
TITLE OF REPORT:	An Evaluation of the Insect Bioefficacy of Combined Trait Products Produced Through Conventional Breeding: MON 89034 x NK603 and MON 89034 x MON 88017
AUTHOR:	Levine, S.L.
STUDY COMPLETED:	July 17, 2006
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study does not meet the GLP requirements specified in 40 CFR Part 1060.
STUDY SUMMARY:	In a laboratory test, the insecticidal activity of corns MON 89034 x MON 88017 and MON 89034 x NK603 was compared to that of the parent corns and a conventional corn. The MON 89034 parent produces Cry1A.105 and Cry2Ab2 insecticidal proteins to control leaf and stalk feeding lepidopterans, while MON 88017 produces Cry3Bb1 protein to provide corn rootworm protection and 5-enolpyruvylshikimate-3-phosphate synthase to provide glyphosate tolerance. NK603 provides glyphosate tolerance. Potted corn plants were infested with either fall armyworm (*Spodoptera frugiperda*) larvae or western corn rootworm (*Diabrotica virgifera*) eggs and evaluated for

	leaf and root damage, respectively, two to three weeks later. In the fall armyworm-infested plants, there were no statistically significant differences in leaf damage ratings between MON 89034 x MON 88017 or MON 89034 x NK603 and the parent corns. In the western corn rootworm plants, there were no statistically significant differences in root damage ratings between MON 89034 x MON 88017 and the parent corns. Combining MON 89034 with either NK603 or MON 88017 via conventional breeding did not alter the insecticidal efficacy of MON 89034 or MON 88017.
CLASSIFICATION:	**Acceptable.**

Test Material

The test materials were MON 89034 x NK603 corn, Lot No. GLP-0411-15626-S, phenotype LPC/RR; and MON 89034 x MON 88017 corn, Lot No. GLP-0411-15628-S, phenotype LPC/CRW/RR.

The test material MON 89034 x NK603 corn is produced by breeding lepidopteran-protected corn MON 89034 with herbicide-tolerant corn NK603. The test material MON 89034 x MON 88017 corn is produced by breeding lepidopteran-protected corn MON 89034 with corn rootworm-protected and herbicide tolerant corn MON 88017. Corn MON 89034 produces Cry1A.105 and Cry2Ab2 proteins, which provide control of damage by certain lepidopteran insect pests. Corn NK603 produces 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which confers tolerance to glyphosate. Corn MON 88017 produces Cry3Bb1 protein, which is coleopteran-active, and EPSPS.

The control substances were MON 89034 corn, Lot No. GLP-0411-15624-S, phenotype LPC; MON 88017 corn, Lot No. GLP-0504-16059-S, phenotype CRW/RR; NK603 corn, Lot No. GLP-0411-15631-S, phenotype RR, and conventional corn, Lot No. GLP-0411-15630-S, phenotype conventional.

The presence or absence of MON 89034, MON 88017, and NK603 in the test and control substance starting seed was stated to be verified by the study sponsor by event-specific polymerase chain reaction analyses. Certificates of analysis were stated to be included in the study file (not provided in MRID 46951304).

Test Methods

The study was conducted to confirm that combining MON 89034 with either NK603 or MON 88017 through conventional breeding does not alter the insecticidal efficacy of MON 89034 or MON 88017. Leaf damage was evaluated on plants infested with larvae of the fall armyworm

(FAW, *Spodoptera frugiperda*), and root damage was evaluated on plant infested with eggs of the western corn rootworm (WCRW, *Diabrotica virgifera*). Each treatment consisted of four replicates of three plants each. The replicates evaluating each insect were arranged in a randomized complete block design.

The test was conducted in a controlled-environment growth chamber with a daytime high temperature of ~28°C, a nighttime temperature of ~24°C, and ~50% relative humidity. The test plants were grown in eight-inch pots (one plant/pot) containing Metro-Mix 200 soil media pre-formulated for growing corn (Hummert International, Earth City, MO). At the V6 growth stage (~2 weeks post-planting) each plant in the FAW test was infested with an estimated 54 first instar FAW larvae, which were allowed to feed for approximately two weeks. After the feeding period, leaf damage ratings of 0-9 (Guthrie et al., 1960) were assigned to each plant. At the V5 growth stage (~2 weeks post-planting) pots in the WCRW test were infested with an estimated 720 WCRW eggs. Approximately three weeks after infestation, damage was evaluated using a nodal injury score of 0.0 to 3.0(Oleson and Tollefson, 2001).

For the FAW test, comparisons of leaf damage were made using PROC MIXED in SAS (v.9.1.3). For the WCRW test, comparisons of root damage were made using the nonparametric procedure PROC NPAR1WAY due to heterogeneity of variance. Separate statistical comparisons were made for the FAW and WCRW tests. All comparisons used a statistical significance level of 5%.

Results Summary

Leaf damage ratings for the FAW test are summarized in Table 1, and statistical results for the corn comparisons are given in Table 2. Mean leaf damage ratings for MON 89034 x NK603, MON 89034 x MON 88017, and MON 89034 were comparable. No significant differences were found in comparisons of mean leaf damage ratings between MON 89034 and MON 89034 x MON 88017 or between MON 89034 and MON 89034 x NK603. Mean leaf damage ratings for MON 88017 and NK603, which do not contain the lepidopteran-protected trait, were comparable to those for conventional corn. As expected, the damage ratings for the latter corns were much greater higher than for the lepidopteran-protected corns.

TABLE 1. Results for fall armyworm leaf damage ratings

Treatment	Phenotype[1]	Mean leaf damage rating ± 95% C. I.
MON 89034 x NK603	LPC/RR	2.75 ± 0.42
MON 89034 x MON 88017	LPC/CRW/RR	3.17 ± 0.54
MON 89034	LPC	2.63 ± 0.68
MON 88017	CRW/RR	8.67 ± 0.23
NK603	RR	9.00 ± 0.00
Conventional	Conventional	9.00 ± 0.00

Data from p. 21, MRID 46951304

[1]LPC = lepidopteran protected corn, RR = Roundup Ready® (glyphosate tolerant), CRW = corn rootworm protected

TABLE 2. Statistical comparison of fall armyworm leaf damage ratings

Comparisons	p-value
MON 89034 x MON 88017 vs MON 89034	0.3505

MON 89034 x MON 88017 vs MON 88017	<0.0001
MON 89034 x MON 88017 vs conventional	<0.0001
MON 89034 x NK603 vs MON 89034	0.9743
MON 89034 x NK603 vs NK603	<0.0001
MON 89034 x NK603 vs conventional	<0.0001

Data from p. 21, MRID 46951304

Nodal injury scores for the WCRW test are summarized in Table 3, and statistical results for the corn comparisons are given in Table 4. Mean nodal injury scores for MON 88017 and MON 89034 x MON 88017, which are corn-rootworm protected, were comparable. The highest nodal injury scores were found in the non-CRW-protected corns. As expected, statistically significant differences were found when comparing the injury scores of MON 89034 x MON 88017 against MON 89034 or conventional corn.

TABLE 3. Results for western corn rootworm nodal injury scores (NIS)

Treatment	Phenotype	Mean NIS ± 95% C. I.
MON 89034 x MON 88017	LPC/CRW/RR	0.29 ± 0.13
MON 89034	LPC	2.23 ± 0.12
MON 88017	CRW/RR	0.18 ± 0.08
Conventional	Conventional	2.03 ± 0.12

Data from p. 22, MRID 46951304

[1]LPC = lepidopteran protected corn, RR = Roundup Ready® (glyphosate tolerant), CRW = corn rootworm protected

TABLE 4. Statistical comparisons for western corn rootworm nodal injury scores

Comparison	p-value
MON 89034 x MON 88017 vs MON 88017	0.3225
MON 89034 x MON 88017 vs MON 89034	<0.0001
MON 89034 x MON 88017 vs conventional	<0.0001

Data from p. 22, MRID 46951304

Study Author's Conclusion

The study author concluded that combining MON 89034 with either NK603 or MON 88017 through conventional breeding did not alter the insecticidal efficacy of either MON 89034 or MON 88017.

Reviewer's Conclusion

BPPD agrees with the study author's conclusion that the efficacy of the stacked MON 89034 x MON 88017 corn product against FAW and WCRW should be comparable to efficacy of the MON 89034 and MON 88017 isolines. However, this conclusion can not be extended to other lepidopteran target pests (including European corn borer, southwestern corn borer, and corn earworm) since they were not evaluated in this study.

References

Guthrie, W.D., F.F. Dicke, and C.R. Neiswander. 1960. Leaf and Sheath Feeding Resistance to the European Corn Borer in Eight Inbred Lines of Dent Corn. Ohio Agricultural Experiment Station Research Bulletin 860.

Oleson, J. and J. Tollefson. 2001. Interactive Node-Injury Scale. Iowa State Entomology internet site. http://www.ent.iastate.edu/pest/rootworm/nodeinjury/nodeinjury.html

DATA EVALUATION RECORD

EPA Reviewer: Alan Reynolds

STUDY TYPE:	Product Performance (Nonguideline)
MRID NO:	470794-03
DP BARCODE:	DP335189
DECISION NO:	339469
SUBMISSION NO:	Not provided
TEST MATERIAL:	MON 89034 x MON 88017
STUDY NO:	05-RA-50-04
SPONSOR:	Monsanto Company 800 N. Lindbergh Blvd. St. Louis, MO 63167
TESTING FACILITY:	Monsanto Company Agronomic Traits 700 Chesterfield Parkway West St. Louis, MO 63017
TITLE OF REPORT:	Assessment of the Efficacy of Insect-protected Corn MON 89034, MON 89034 x NK603 and MON 89034 x MON 88017 Against Major Insect Pests in the Field Trials Conducted in the U.S. During 2005
AUTHOR:	Headrick, J., Heredia, O., Oyediran, I.
REPORT DATE:	August 31, 2006
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study does not meet the GLP requirements specified in 40 CFR Part 1060.
STUDY SUMMARY:	Field trials in 14 locations in 9 states were conducted to evaluate the efficacy of MON 89034 x MON 88017 corn against 7 lepidopteran pests: European corn borer, southwestern corn borer, corn earworm, fall armyworm, sugarcane borer, western bean cutworm, and black cutworm. Artificial and natural infestations were used and efficacy was determined by assessing feeding damage. For ECB, SWCB, FAW, and CEW the efficacy of MON 89034 x MON 88017 was comparable to MON 89034. Both MON 89034 and MON 89034 x MON 88017 provided equivalent or better efficacy than MON 810 against these target pests. MON 89034 was equivalent to

	MON 810 for SCB. Separate trials for WBCW and BCW showed that MON 89034 was more efficacious than MON 810, although limited locations were tested. It is noted that SCB, WBCW and BCW trials did not include MON 89034 x MON 88017 as a treatment, so efficacy could not be directly evaluated. Overall, MON 89034 x MON 88017 can be expected to provide comparable control to MON 89034 against lepidopteran pests. Further, both MON 89034 and MON 89034 x MON 88017 should control a broader range of lepidopteran pests than MON 810.
CLASSIFICATION:	**Acceptable.**

Test Material

The test materials for the experiments included two stacked hybrids: MON 89034 x NK603 corn and MON 89034 x MON 88017 corn. Also tested were single toxin isolines including MON 89034 corn, MON 88017 corn, MON 810 corn, NK603 corn, and conventional (non-engineered) corn. Lot numbers were not provided for any of the hybrids.

The test material MON 89034 x NK603 corn is produced by breeding lepidopteran-protected corn MON 89034 with herbicide-tolerant corn NK603. The test material MON 89034 x MON 88017 corn is produced by breeding lepidopteran-protected corn MON 89034 with corn rootworm-protected and herbicide tolerant corn MON 88017. Corn MON 89034 produces Cry1A.105 and Cry2Ab2 proteins, which provide control of damage by certain lepidopteran insect pests. Corn NK603 produces 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which confers tolerance to glyphosate. Corn MON 88017 produces Cry3Bb1 protein, which is coleopteran-active, and EPSPS while MON 810 expresses Cry1Ab protein for control of lepidopteran pests.

Conventional soil insecticides (Poncho 250, Poncho 1250, and Force) were included in some of the trials as an additional test standard.

Test Methods

A series of field trials were conducted to evaluate efficacy against seven lepidopteran corn pests, including: European corn borer (ECB, *Ostrinia nubilalis*), southwestern corn borer (SWCB, *Diatraea grandiosella*), corn earworm (CEW, *Helicoverpa zea*), fall armyworm (FAW, *Spodoptera frugiperda*), sugarcane borer (SCB, *Diatraea saccharalis*), western bean cutworm (WBCW, *Richia albicosta*), and black cutworm (BCW, *Agrotis ipsilon*). Both natural and artificial infestations were used in the tests and each treatment consisted of four replicates arranged in a randomized complete block design. The replicates consisted of one to four row plots containing 35 plants per row (total row length 5.3 to 6.1 m). Fourteen total locations in nine states were used for the experiments, although not all pests were evaluated at each site. Table 1 below lists the use sites, infestation method and pests evaluated.

Table 1. Summary of the U.S. field efficacy trials conducted during 2005 (taken from Table 1 in MRID# 470794-03)

Location	Infestation method	Targeted Insect Pests						
		ECB	SWCB	FAW	CEW	SCB	BCW	WBCW
WI-A	Artificial	√						
IL-W	Artificial	√					√	
IL-M	Artificial	√			√		√	
IL-J	Artificial	√			√		√	
MO-M	Artificial	√					√	
TN-UC	Artificial		√	√	√			
TN-M	Artificial		√		√			
MS-L	Artificial		√	√				
AL-L	Artificial		√	√				
LA-C	Natural					√		
NE-I	Both							√
CO-K	Both							√
CO-S	Both							√
CO-Y	Both							√

Specific notes on infestation methods and efficacy assessments for each pest are described below:

ECB

The trials for ECB were conducted at five total locations in Wisconsin, Illinois, and Missouri. Artificial infestation of 50 neonate larvae per plant was used with a "Bazooka" applicator. First generation ECB infestation was simulated by infesting plants at the V8 growth stage. A second infestation at the mid silk stage was made to simulate second generation ECB. Efficacy was evaluated by leaf damage ratings employing the Guthrie 0 (least damaged) to 9 (most damaged) scale. Stalk tunneling was also measured (in cm) for the second generation infestation.

SWCB

Artificial infestations of 70 neonates per plant using a Bazooka applicator were made at four total locations in Tennessee, Mississippi, and Alabama. The same procedures that were used with ECB were also used with SWCB to simulate first and second generation infestations. Feeding damage (0-9 Guthrie scale) and stalk tunneling were assessed in the tests.

SCB

One location in Louisiana was tested for SCB using natural infestations (reported as heavy insect pressure). Three hybrids were tested: MON 89034, MON 810, and a non-Bt

control. Plant damage was assessed by splitting corn stalks lengthwise and measuring tunneling created by SCB larvae.

FAW

FAW were evaluated at three total locations in Tennessee, Mississippi, and Alabama. Corn plants (V8 stage) were artificially infested with 50 neonate larvae per plant with a Bazooka applicator. In cases were the initial infestation was adversely affected by weather, repeat infestations were applied. Feeding damage was assessed by sampling 10 plants in each plot and using the 0-9 Davis scale.

CEW

Testing for CEW was conducted at four total locations in Illinois and Tennessee using artificial infestation. Fifteen neonate larvae per plant were applied to the green silks of corn plants with a subsequent application two to three days later. Ten ears per plot were sampled and feeding damage was assessed with the Widstrom scale (0 = no damage, 1 = damage to silks, and n + 1 for ear/kernel damage of n cm.

WBCW

WBCW were tested at four locations in Colorado and Nebraska using both natural and artificial infestation. Egg masses collected from nearby bean fields were used in the artificial infestations (25 per plot, stapled to leaves below the primary ear) to augment the natural insect pressure. Each corn ear in the test plots was examined for WBCW larvae and 30 ears were sampled for feeding damage. CEW were also found in the test plots and were also tabulated during the experiment.

BCW

For BCW, efficacy tests were conducted at four total locations in Illinois and Missouri using artificial infestation. Large larvae (third or early fourth instar) were applied to the tests plots (two per plant, positioned near the base of V1 corn plants) with iron barriers to prevent escape of the larvae. Damage was determined by counting the number of plants clipped at 1, 3, 7, and 14 day observation periods.

Results Summary

The efficacy results for each of the tested target pests are discussed individually below.

ECB

Among the tested hybrids, events with MON 89034 (including MON 89034, MON 89034 x NK603, and MON 89034 x MON 88017) or MON 810 (single gene) had significantly less ECB

feeding damage than hybrids without lepidopteran active toxins (i.e. MON 88017, NK603, or non-Bt corn). All of the MON 89034 (Cry1A.105 and Cry2Ab2) hybrids had Guthrie leaf damage ratings of <1 (MON 810 was ~ 1.5) while the non-lepidopteran active hybrids had ratings of ~ 3-4. Stalk tunneling patterns with similar with the MON 89034 hybrids and MON 810 exhibiting average tunneling of <1 cm with the non-lepidopteran events showing average tunneling of at least 3 cm.

The study authors noted low overall ECB feeding in the trials (leaf damage scores on the non-Bt controls were approximately 3-4 on the 0-9 Guthrie scale). However, the feeding differences between MON 89034/MON 810 hybrids were statistically different than the other tested hybrids when analyzed using a pair-wise t-test. Results from the experiments are displayed in Figures 1 and 2 below.

Figure 1. Efficacy of MON 89034, MON 89034 x MON 88017, and MON 89034 x NK603 against ECB first generation: assessment of leaf feeding damage. Results presented as a mean of three locations. Values indicated by the same letter are not statistically different at $\alpha = 0.05$ level. (Taken from Figure 1 in MRID# 470794-03.)

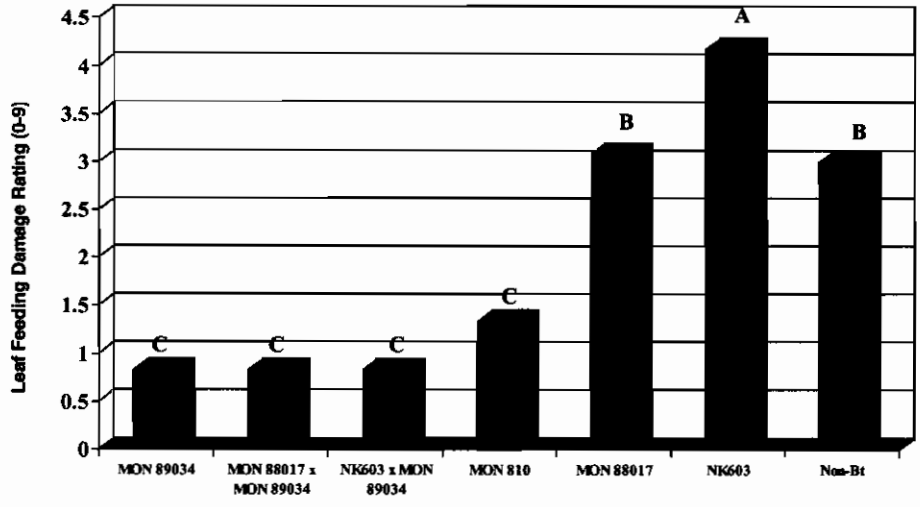
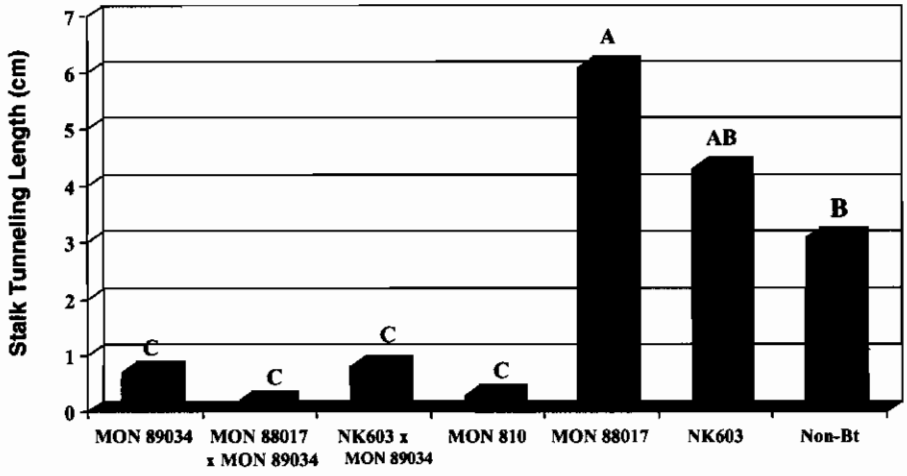


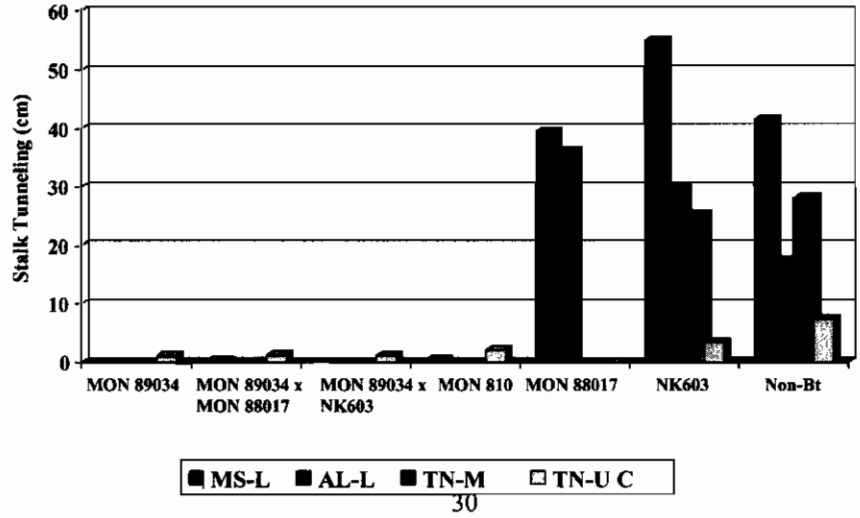
Figure 2. Efficacy of MON 89034, MON 89034 x MON 88017, and MON 89034 x NK603 against ECB second generation: assessment of stalk tunneling length. Results presented as a mean of four locations. Values indicated by the same letter are not statistically different at $\alpha = 0.05$ level. (Taken from Figure 2 in MRID# 470794-03.)



SWCB

Efficacy patterns with SWCB were similar to those observed in the ECB trials: MON 89034, MON 89034 x NK603, MON 89034 x MON 88017, and MON 810 had significantly less stalk tunneling damage than the control hybrids (i.e. MON 88017, NK603, or non-Bt corn). The MON 89034/MON 810 hybrids had virtually no tunneling damage while the non-lepidopteran hybrids had average tunnel lengths of 4.7 to 59.7 cm. Leaf feeding damage data was not reported in the submission -- although the description of methods indicated leaf damage would be assessed, it is unclear if these data were ever collected. The results from the SWCB trials are shown in Figure 3 below.

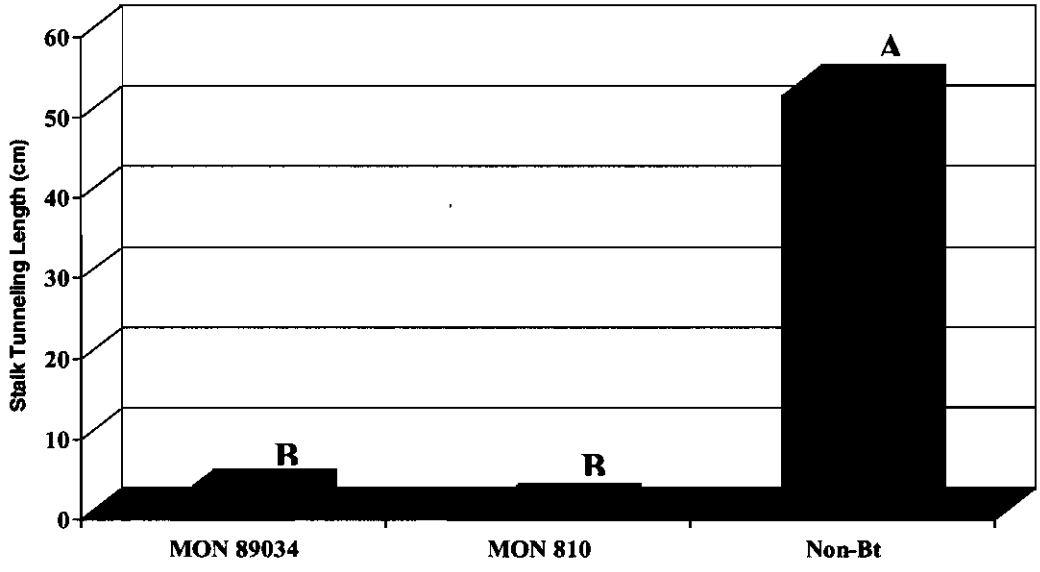
Figure 3. Efficacy of MON 89034, MON 89034 x MON 88017, and MON 89034 x NK603 against SWCB: assessment of stalk tunneling length by location. (Taken from Figure 3 in MRID# 470794-03.)



SCB

In a trial with heavy natural infestation, both MON 89034 and MON 810 had significantly less stalk tunnel damage (≤ 2.3 cm) than the non-Bt control (52.5 cm). Stacked and herbicide tolerant hybrids were not tested as part of the SCB evaluation. The results are summarized in Figure 4 below.

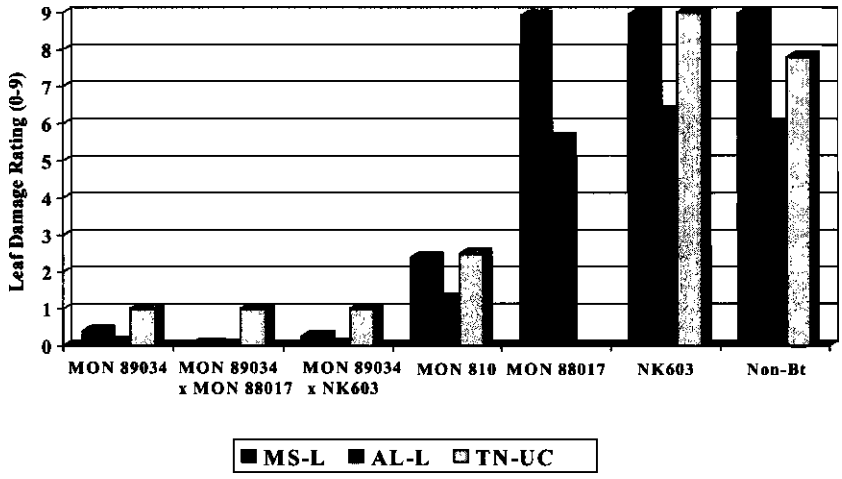
Figure 4. Efficacy of MON 89034 against SCB: assessment of stalk tunneling length at LA-C location. Values indicated by the same letter are not statistically different at $\alpha = 0.05$ level. (Taken from Figure 4 in MRID# 470794-03.)



FAW

MON 89034 containing events (MON 89034, MON 89034 x NK603, and MON 89034 x MON 88017) had lower FAW leaf feeding damage than MON 88017, NK603, and non-Bt corn. Leaf damage ratings for the MON 89034 hybrids were 1 or lower on the 0-9 Davis scale, while the ratings for the non-lepidopteran targeted events were 5 or greater. MON 89034 also performed better than MON 810, which had damage ratings of ~ 1 to 2.5. Statistical analyses were not presented with the data and it is unclear whether the differences between MON 89034 and MON 810 are statistically significant. The results are shown in Figure 5 below.

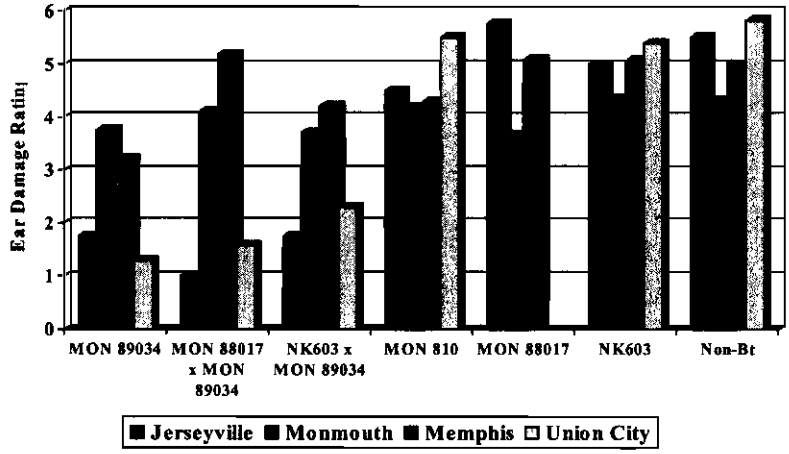
Figure 5. Efficacy of MON 89034, MON 89034 x MON 88017, and MON 89034 x NK603 against FAW: assessment of leaf feeding damage by location. (Taken from Figure 5 in MRID# 470794-03.)



CEW

Hybrids containing MON 89034 (MON 89034, MON 89034 x NK603, and MON 89034 x MON 88017) had significant less corn ear feeding damage than both MON 810, MON 88017 and the non-Bt control. However, some damage was still noted in the MON 89034 groups, with Widstrom ear damage ratings ranging from 1 to 5 (damage ratings for the other groups were ~ 3.5 to 6). High variability between treatment locations was noted, possibly due to erratic feeding by CEW. The results of the CEW trials are shown in Figure below.

Figure 6. Efficacy of MON 89034, MON 89034 x MON 88017, and MON 89034 x NK603 against CEW: assessment of ear damage by location. (Taken from Figure 6 in MRID# 470794-03.)



WBCW

WBCW feeding damage (arising from natural infestation with supplemental artificial infestations) was significantly lower on MON 89034 (2% ear damage) than either MON 810 (4.8%) and the non-Bt control (5.4%). There was low overall pest pressure from WBCW, although low variability between locations was noted. The feeding damage results are summarized in Figure 7 below.

At one location, the presence of CEW was noted as well. Greater ear damage was noted for all treatments at this location, although with similar trends as the other locations: MON 89034 had significantly less damage (3.3%) than MON 810 (~ 12%) and non-Bt corn (15%). The results for this location are contained in Figure 8 below.

It is noted that none of the stacked hybrids (i.e. MON 89034 x NK603, and MON 89034 x MON 88017) were tested in this experiment.

Figure 7. Efficacy of MON 89034 against WBCW: assessment of ears damaged by the incidence of WBCW larvae present on a plant. Results presented as a mean of four locations. Values indicated by the same letter are not statistically different at $\alpha = 0.05$ level. (Taken from Figure 7 in MRID# 470794-03.)

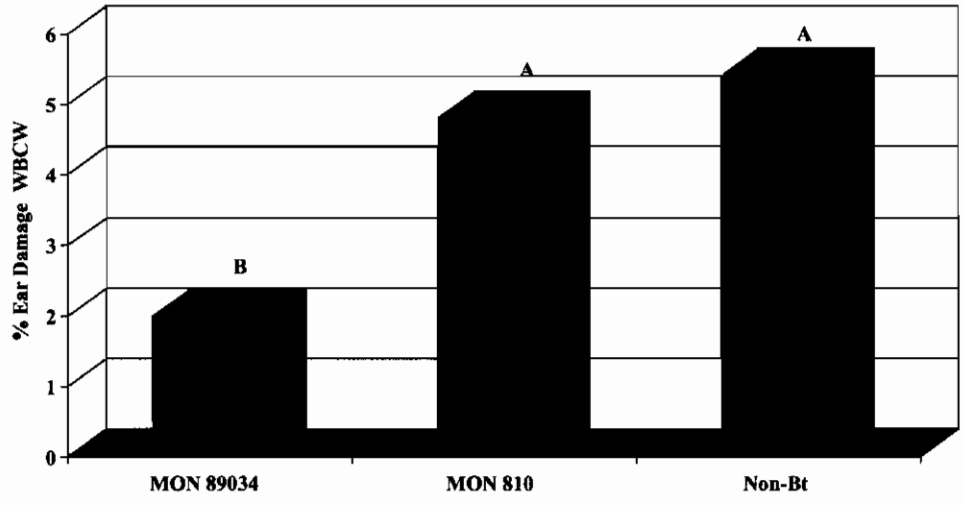
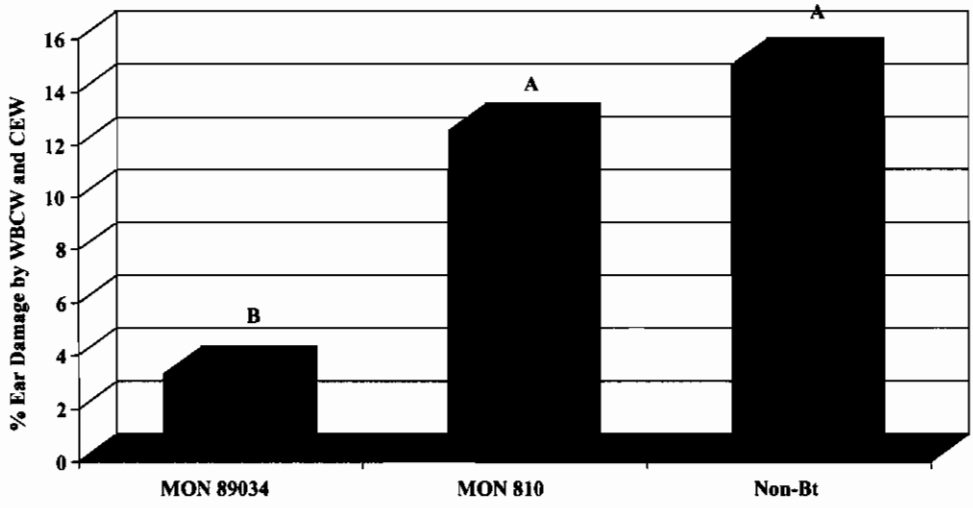


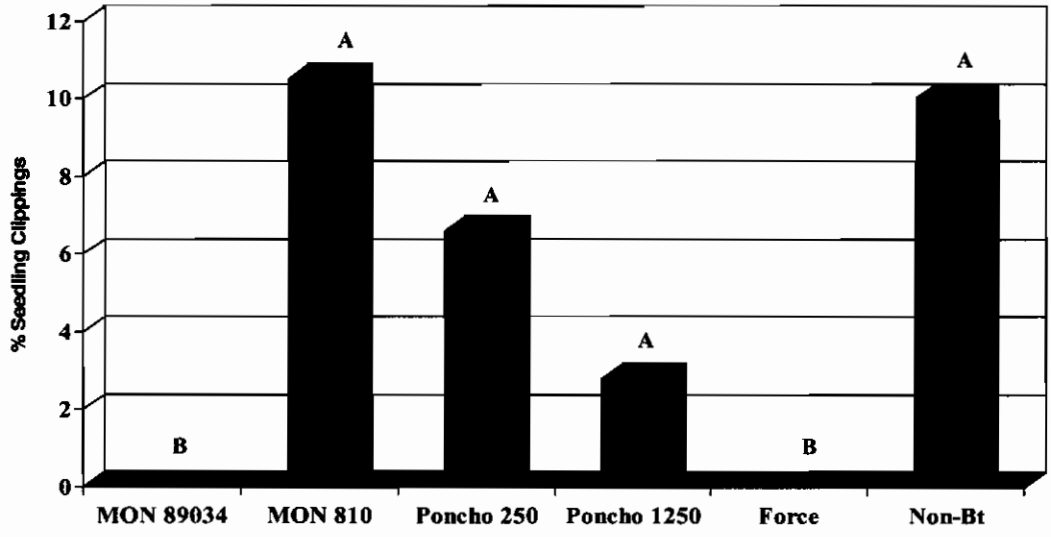
Figure 8. Efficacy of MON 89034 against dual infestation of WBCW and CEW: assessment of ears damaged by the incidence of WBCW and CEW larvae present on a plant at CO-Y location. Values indicated by the same letter are not statistically different at $\alpha = 0.05$ level. (Taken from Figure 8 in MRID# 470794-03.)



BCW

Although four locations were tested for BCW efficacy, only one location was reported due to high variability in the results. At this one location, none of the MON 89034 seedlings showed clipping damage from BCW, comparable to the conventional insecticide treatment Force (no damage). The other treatments including MON 810 (10% clipped seedlings), non-Bt corn (~ 10%), and conventional insecticides Poncho 250 (6.6%) and Poncho 1250 (2.8%) had greater BCW damage. Stacked hybrids (i.e. MON 89034 x NK603, and MON 89034 x MON 88017) were not included in this experiment. The results are displayed in Figure 9 below.

Figure 9. Efficacy of MON 89034 against BCW: assessment of seedling clippings compared to commercial insecticides at MO-M location. Values indicated by the same letter are not statistically different at $\alpha = 0.05$ level. (Taken from Figure 9 in MRID# 470794-03.)



Study Author's Conclusion

Monsanto concluded that the efficacy of MON 89034 and MON 89034 x MON 88017 was equivalent or better to MON 810 for ECB, SWCB, CEW, and FAW. MON 89034 also provided better control than MON 810 of SCB, WBCW, and BCW, although limited locations were tested and additional data would be needed to verify the results. Overall, the company noted that MON 89034 provides a broader range of activity against lepidopteran pests than MON 810.

Reviewer's Conclusion

BPPD agrees with the study author's conclusion that the efficacy of the stacked MON 89034 x MON 88017 corn product against ECB, SWCB, CEW, and FAW should be comparable to efficacy of the MON 89034 isoline. There were no statistical differences in pest damage between MON 89034 and MON 89034 x MON 88017 and both hybrids significantly reduced damage relative to the non-lepidopteran active and non-Bt control groups. Additionally, the data support the conclusion that both MON 89034 and MON 89034 x MON 88017 provide a broader spectrum of efficacy than MON 810 against lepidopteran pests. The SCB, WBCW and BCW trials did not include MON 89034 x MON 88017 as a treatment, so efficacy could not be directly evaluated. However, given the performance of MON 89034 x MON 88017 against the other target pests, it is reasonable to assume comparable efficacy to MON 89034 for these pests as well. BPPD also notes that WBCW and BCW are considered secondary pests of corn for most U.S. corn-growing regions.

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Chemical: Bacillus thuringiensis Cry3Bb1 protein and the genetic material necessary (vector ZMIR39) for its production in corn
Bacillus thuringiensis Cry1A.105 protein and genetic material necessary (vector PV-ZMIR245) for its production in corn
Bacillus thuringiensis Cry2Ab2 protein and the genetic material necessary (vector PV-ZMIR245) for its production in corn

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HED File Code: 41400 BPPD IRM
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File ID: DPD335189
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