

US EPA ARCHIVE DOCUMENT

MEMORANDUM

Subject: Preliminary review of Monsanto's interim insect resistance management plan for *Bacillus thuringiensis* event MON 863 corn rootworm protected field corn. EPA Reg. No. 524-LEI; Chemical No. 006484; Barcode No. D280086; Case No. 069017; Submission No. S607630; MRID Nos. 455770-01, 455382-08 and 451568-05.

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Classification: It is **acceptable** to plant MON 863 corn for three years with a 20% non-Bt corn refuge planted within or adjacent to Bt corn fields. Based on the limited acres, use of a refuge and three year time frame, the risk of pest resistance to MON 863 is not a concern.

RECOMMENDATIONS

- Based on current information on CRW biology, MON 863 dose, simulation models, hybrid availability and adoption rate, a 20% refuge should be adequate on an interim basis to produce enough CRW adults to delay resistance. In addition, planting refuges adjacent to or within MON 863 corn fields should allow random mating of CRW even though there is limited movement of larvae and virgin females.
- It is acceptable to plant refuges as continuous blocks or in-field row-strips. Based on the only available currently published data, in-field strips should consist of at least 6 to 12 consecutive rows planted within 9 to 18 m of the center of the transgenic corn field.
- WCRW, NCRW and MCRW only feed on a few weedy species other than corn and the value of these alternate hosts as a refuge is not understood (Meinke *et al.* 2001). Therefore, alternate hosts should not be considered as refuge for CRW-protected transgenic corn until

more information is available on their ability to produce susceptible individuals available to mate with potentially resistance beetles.

- Additional information is needed on various aspects of CRW pest biology as it relates to a long-term IRM strategy. Knowledge of the maximum and average distance an adult CRW moves is limited. Additional research regarding male and female adult and larval WCRW and NCRW dispersal potential is needed to determine placement of non-Bt corn refuges. In addition, more information is needed on mating habits, ovipositional patterns, number of times a female can mate and fecundity.
- Additional research is needed to determine if IRM strategies designed for WCRW and NCRW are appropriate for MCRW and if IRM is needed for SCRW. Since MON 863 expression in corn roots likely declines as the plant matures and MCRW may develop slower, the risk of resistance may differ for MCRW then WCRW and NCRW.
- The mechanism of potential resistance of CRW to MON 863 should be determined to develop an appropriate long-term IRM strategy. Since CRW resistance is necessary to determine the mechanism and genetics of resistance to Cry3Bb1, colonies resistant to Bt should be established and evaluated in the laboratory during the initial three years MON 863 is grown commercially.
- The effect of WCRW ovipositing in soybean prior to overwintering and extended diapause in NCRW on an IRM strategy needs further investigation.
- Based on Monsanto's modified version of Caprio's model, a moderate dose is defined as 30% survival of larvae and a low dose as 50% survival. Data provided by Monsanto shows 17% to 62% survival of larvae, which suggests that there is a low to moderate dose of MON 863 for CRW. "Further research is needed to provide the critical data on the actual dose effects of Bt corn, corn rootworm biology and ecology" (Ostlie 2001).
- Additional knowledge of pest biology, population dynamics and genetics are necessary for the models needed to develop and validate resistance management strategies for CRW-protected corn.
- Resistance management models need further refinement and validation. Monsanto primarily relies upon a modified version of Mike Caprio's model which was originally developed for lepidopteran-active corn (ILSI 1999). Investigation into the validity of this model for coleopteran-protected corn is needed. Additional models should be developed that consider parameters such as seed mixtures, in-field refuge configurations, behavioral avoidance and fitness. Assumptions of simulation models should be tested and validated in the field.
- Grain producers will utilize the option of applying insecticides to treat for CRW adults or larvae on refuge acres because of high economic loss if left untreated. Insecticides are applied to the root zone to control larvae and will not affect CRW out of the treated area (Meinke *et al.* 2001). Therefore, it should be assumed that refuge acres will be treated for CRW larvae with chemical insecticides. However, insecticides should not be used on

refuges to control CRW adults. If resistance is confirmed, all acres (Bt fields and non-Bt refuges) should be treated with insecticides targeted at CRW adults as well as larvae.

- A resistance monitoring strategy for CRW-protected Bt corn is needed to test the effectiveness of resistance management programs. Detecting shifts in the frequency of resistance genes (i.e., susceptibility changes) through resistance monitoring can be an aggressive method to detect the onset of resistance before widespread crop failure occurs. As such, the utilization of sensitive and effective resistance monitoring techniques is critical to the success of an IRM plan. Baseline susceptibility studies for WCRW have been initiated from populations collected in 1999 and 2000 by Dr. Blair Siegfried (University of Nebraska), Dr. Larry Chandler (USDA-ARS) and Dr. Wade French (USDA-ARS). These studies should be continued for WCRW and initiated for NCRW and monitoring techniques such as discriminating dose concentration assays need to be thoroughly investigated for their feasibility as resistance monitoring tools.
- There is a concern regarding Monsanto's proposed outline of detecting and confirming resistance. Monsanto suggests that they will initiate mitigation measures when unexpected levels of CRW damage occur. However, Monsanto does not describe what is meant by unexpected levels of damage. Some level of damage is expected since there is not a high dose of MON 863 expressed to control the CRW and research has shown that some level of "grazing" will occur. Since confirmation of resistance is time-consuming, an interim remedial action (mitigation) plan should be implemented as soon as resistance is suspected. Monsanto must identify what unexpected levels of damage are and relay this information to EPA, seed dealers, extension specialists, NCGA and growers. Monsanto should also develop an remedial action plan that is approved by the Agency.

BACKGROUND

Monsanto Co. has submitted an application to EPA for the registration of *Bacillus thuringiensis* (Bt) Cry3Bb1 protein and the genetic material (Vector ZMIR13L) necessary for its production in corn. Corn expressing the Cry3Bb1 protein is intended to provide protection against the corn rootworm (CRW, *Diabrotica* spp.). This product has been designated event MON 863 by Monsanto. Prior to registering Cry3Bb1 corn, an acceptable insect resistance management (IRM) plan is necessary. Monsanto designed a plan intended to be scientifically valid for resistance risk mitigation and feasible for growers to understand, implement and comply with. Since Monsanto acknowledges that a robust and practical IRM plan will require time to develop, they are proposing a three-year interim plan. An interim plan was submitted by Monsanto because they believe growers need to be able to grow MON 863 corn for a period of time so that important information can be generated and growers are provided an understanding of IRM requirements.

Monsanto submitted several documents in support of their proposed IRM plan. An IRM plan for MON 863 corn dated June 20, 2000 was submitted to the Agency (MRID No. 451568-05). This submission included information on dose, CRW biology, simulation models of resistance development, and grower surveys. Research reports and results of grower surveys were also included in the Appendices of the June 2000 submission. An amended IRM plan dated January

8, 2002 was submitted to the Agency for review (MRID No. 455770-01). The amended plan titled "An Interim Insect Resistance Management Plan for Corn Event MON 863: A Transgenic Corn Rootworm Control Product" was intended to supercede MRID No. 451568-05. Therefore, MRID No. 451568-05 was used for additional information and as reference material, but was not formally reviewed. An additional preliminary research report dated February 20, 2001 was submitted to the Agency by Monsanto (MRID No. 453484-01). Although this report is preliminary and was not formally reviewed, information found in MRID No. 453484-01 was considered in evaluating Monsanto's proposed interim IRM plan. A review in the form of a data evaluation report (DER) of Monsanto's interim IRM plan found in MRID No. 455770-01 is attached to this memorandum.

CONCLUSIONS

Currently, there is not enough information available to definitively determine the size and structure of a non-Bt corn refuge for MON 863 needed to appropriately delay the development of CRW resistance. In a memorandum dated April 12, 2002 and e-mailed to the Agency by Russ Schneider (Monsanto Co.), the Canadian Food Inspection Agency (CFIA) states that the current Monsanto IRM plan is based on a high dose product for ECB control; however, MON 863 does not provide a high dose. Therefore, the CFIA concluded that "[i]t is currently uncertain whether requirements for refuge placement and the proportion of field planted to a refuge will be similar or different to that in place for ECB hybrids. The Agency acknowledges that there is a lack of information on dose and pest biology; therefore, a conservative approach to CRW IRM is necessary. Current information suggests that MON 863 provides a low to moderate dose of Cry3Bb1 for CRW.

The Agency agrees with the following recommendations quoted from the CFIA April 12, 2002 memo. "1. The refuge of a non-Bt corn hybrid consists of no less than 20% of the total corn planted in a field. 2. The refuge and Bt planting areas should both have the same crop rotation histories. 3. The refuge is located within a field with a flexible configuration of non-Bt rows planted to convenience the grower and orientation of the field. 4. Where circumstances require that a refuge cannot be planted within a field, the refuge must be planted adjacent to the Bt field. 5. The refuge may be treated with a banded soil insecticide for larval rootworm control."

Based on current information on CRW biology, MON 863 dose, simulation models, hybrid availability and adoption rate, a 20% refuge should be adequate to produce enough CRW adults to delay resistance. In addition, planting refuges adjacent to or within MON 863 corn fields should allow random mating of CRW even though there is limited movement of larvae and virgin females.

Monsanto expects the number of acres of MON 863 corn will be limited in each state during the first three years of registration because of a lack of hybrid availability and slow grower adoption rates as they evaluate the new technology. Therefore, Monsanto suggests that there will potentially be a greater than 20% refuge leading to a decrease in the risk of resistance. Although Monsanto does not plan to plant a refuge for inbred seed increase fields, they will treat with insecticides that will probably control any resistant CRW adults that may emerge. It is, therefore, acceptable to plant inbred seed increase acres without a refuge.

Planting a limited number of acres per state of MON 863 corn with a 20% non-Bt corn refuge planted adjacent to the Bt field or as strips within the field should be adequate to ensure resistance does not develop during the three year interim period requested by Monsanto. Although unmated adult CRW females have been shown to move up to ten rows, it may not be practical for growers to plant ten row strips. Therefore, at least six rows (but preferably 12 rows) of non-Bt corn should be planted when in-field strips are utilized as refuge (Onstad *et al.* 2001). In addition, refuges should be planted with similar hybrids as the MON 863 field and identical agronomic practices should be employed for the transgenic and non-transgenic fields to ensure simultaneous adult emergence.

The NCR-46 committee which consists of research and extension CRW specialists as well as other cooperators commented on Monsanto's interim IRM plan at the request of the BPPD IRM Team. This group is recognized at the national authorities on CRW biology, ecology and management. The NCR-46 agrees that the potential for CRW to develop resistance to MON 863 during a three year interim period with low adoption rates is negligible. Since there is not a high dose of MON 863, there will be a significant number of CRW larvae survival. In addition, evidence of changes in larval feeding behavior on MON 863 corn will lead to increased larval survivorship. This increase in larval survivorship will result in a decreased chance of resistance.

Monsanto claims that MON 863 controls CRW (*Diabrotica* spp.). This implies that all CRW species including WCRW, NCRW, MCRW and SCRW will be controlled by MON 863. However, in their "interim IRM plan", Monsanto does not address the Mexican corn rootworm or southern corn rootworm. Information on pest biology and refuge structure is needed for a general claim of *Diabrotica* spp. control. Additional information is needed on various aspects of CRW pest biology as it relates to an IRM strategy.

Monsanto also does not adequately address grower education or mitigation measures if resistance occurs. A detailed grower education program is necessary to ensure grower compliance, particularly since the IRM requirements may change as more information becomes available. Growers should be required to sign a Technology/Stewardship Agreement each year they intend to plant MON 863 corn to ensure they are aware of the current IRM requirements.

There is also concern regarding Monsanto's outline of detecting and confirming resistance. Monsanto suggests that they will initiate mitigation measures when unexpected levels of CRW damage occur. However, Monsanto does not describe what is meant by unexpected levels of damage. Some level of damage is expected since there is not a high dose of MON 863 expressed to control the CRW and research has shown that some level of "grazing" will occur. Monsanto must identify what unexpected levels of damage are and relay this information to EPA, seed dealers, extension specialists, NCGA and growers. This may be accomplished in many ways including websites, a 1-800 telephone number and Technology Use Guides. Further investigation into confirming resistance is also needed. Monsanto's time-line includes identifying the insects as resistant, confirming the plant is expressing MON 863 and taking mitigation measures within 90 days. After this length of time, the growing season will likely be over and it will be too late for mitigation measures. It is, therefore, necessary for Monsanto to cease sales in an affected area after a confirmed report of resistance. The area and amount of time (in years) that sales should cease should be determined in consultation with EPA.

In addition, pages 6 and 9 of the IRM submission states that “[h]ybrids containing event MON 863 will be released both as stand-alone products and in combination with the *cry1Ab* gene for lepidopteran control because of the combined insect management needs of many farmers.” However, the risk of resistance from a Cry1Ab/Cry3Bb stacked product has not been determined. Prior to the release and distribution of a Cry1Ab/Cry3Bb1 “stacked” product, an acceptable IRM plan specifically designed for a “stacked” product must be submitted to the Agency for review. Although refuges are similar for the lepidopteran protected and coleopteran protected Bt corn, Monsanto should consult with the Agency prior to distributing combination products.

DATA EVALUATION REPORT

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STUDY TYPE: Insect Resistance Management Plan

MRID NO.: 455770-01

STUDY NO.: MSL-17556

SPONSOR: Monsanto Company, 700 Chesterfield Parkway North, St
 Louis, MO 63198

TEST MATERIAL: *Bacillus thuringiensis* (Bt) corn event MON 863

AUTHOR: Ty Vaughn, Dennis Ward, Jay Pershing, Graham Head, and
 John McFerson

STUDY COMPLETED: December 15, 2001

CLASSIFICATION: No classification since this is a non-guideline study

Title: “An Interim Insect Resistance Management Plan for Corn Event MON 863: A Transgenic Corn Rootworm Control Product:

Authors: Ty Vaughn, Dennis Ward, Jay Pershing, Graham Head, and John McFerson

Objective: This plan was designed by Monsanto to ensure that the risk of CRW developing resistance to MON 863 corn is “insignificant” during a three-year interim period.

Summary of the General Elements of Monsanto’s Proposed Interim IRM Plan:

This section summarizes Monsanto’s discussion of their proposed insect resistance management (IRM) plan summarized in MRID No. 455770-01. Monsanto has developed an IRM plan that they believe will be cost-effective, flexible, easily adopted by growers and compatible with common production practices. They are requesting that this plan be considered an interim IRM plan for *Bacillus thuringiensis* (Bt) event MON 863 corn rootworm-protected corn. Under Monsanto’s proposal, this IRM plan would be implemented for three years while additional research is conducted to determine an acceptable long-term strategy to mitigate corn rootworm (CRW; *Diabrotica* spp.) resistance to MON 863 (Table 1). Monsanto intends for this IRM plan to evolve over time as new information becomes available. In their submission, Monsanto suggests planting a 20% non-Bt corn refuge planted within or adjacent to MON 863 fields to encourage random mating of CRW.

Monsanto expects negligible selection pressure for resistance during the first few years after MON 863 is sold commercially because low initial adoption rate is anticipated due to limited hybrid availability and grower characteristics. Availability of MON 863 to growers will be low initially because of production constraints in converting inbred lines used in the production of commercial hybrids. In addition, grower adoption is expected to be slow while this technology

is new and they need to determine how it will fit into their current farming practices. Monsanto also intends to reduce the risk of resistance by encouraging stakeholders such as growers, Extension Agents and crop consultants to promote and practice integrated pest management (IPM). For instance, scouting fields in the Fall will be encouraged and planting MON 863 on acres with the highest risk of damage will be promoted.

In Monsanto's plan, growers will be encouraged to manage refuges to promote adequate beetle survival and CRW fecundity. For instance, Monsanto suggests that refuges should promote moderate to high CRW populations because decreasing the number of larvae should lead to an increase in the number of adults emerging. This may be accomplished by planting insecticide treated seed or through soil insecticide application. Adult emergence in refuges will be extended by planting a mid to full-season hybrid. Also, beetle survival can be improved in refuges by planting large blocks or adjacent fields prior to egg hatch and away from areas prone to flooding or standing water.

Table 1. List of research currently underway sponsored by Monsanto

Study Title	Author(s)	Relevance to IRM
Post-establishment movement of western corn rootworm larvae - three years of data under Missouri conditions	Bruce E. Hibbard	*Pest Biology & Refuge Placement
Tracking western corn rootworm pre-mating movement	Doug Tallamy	*Pest Biology and Refuge Placement
Efficacy of between field refuges for resistance management of transgenic corn rootworm-insecticidal corn	David Andow & Donald Alstad	*Simulation Models and Refuge Placement
Modeling the dynamics of adaptation to transgenic corn by western corn rootworm	David Onstad***	*Simulation Models
An economic model of CRW protected transgenic corn IRM strategies	Terry Hurley	*Simulation Models
An economic model of CRW protected transgenic corn IRM strategies	John Foster	*Pest Biology
Resistance management for western corn rootworm (Coleoptera: Chrysomelidae) using seed mixtures of transgenic corn expressing the Cry3B protein	Wade French	*Refuge
Resistance management for western corn rootworms (Coleoptera: Chrysomelidae) using seed mixtures of transgenic corn expressing the Cry3B protein	Elson Shields	*Refuge
Resistance management for western corn rootworms (Coleoptera: Chrysomelidae) using seed mixtures of transgenic corn expressing Cry3B protein	Dennis Calvin	*Refuge
Impact of transgenic corn rootworm protected corn on selected western corn rootworm life history traits	Lance Meinke	*Dose
Flight characteristics of female western corn rootworms exposed to transgenic plants	Jon Tollefson & Ted Wilson	*Pest Biology & Refuge Placement

Study Title	Author(s)	Relevance to IRM
Efficacy of Monsanto's lead transgenic events resistant to corn rootworm larvae feeding: root damage ratings and adult emergence	Jon Tollefson <i>et al.</i>	*Dose
An evaluation of CRW dispersal and mating behavior	Joseph Spencer	*Pest Biology & Refuge Placement
Estimating base line susceptibility in natural population of CRW	Blair Seigfried	*Resistance Monitoring
Estimating the impact of foxtail on CRW populations	Ken Ostlie Mike Gray	*Refuge
Larval feeding behavior of <i>Diabrotica virgifera virgifera</i> : progress report	Pete Clark & John Foster	**Pest Biology
An evaluation of Cry3Bb expression and impact on western corn rootworm from transgenic events	Vaughn <i>et al.</i>	**Dose
Efficacy of advanced transgenic corn rootworm events against CRW pest species - results of year 2000 & 2001 trials	DeGooyer	**Dose
1999 evaluation of Monsanto transgenic corn germplasm for corn rootworm larval feeding resistance and adult emergence	Hibbard	**Dose
Impact of CRW-protected corn on silk clipping & beetle numbers	Davis, Pilcher & Spangler	**Dose

* Research summarized in MRID 453484-01

** Research summarized in Appendix C of MRID 455770-01

***This paper is now published in Onstad *et al.* 2001

Pest Biology:

Summary of Monsanto's Proposal

Monsanto discusses pest biology in Section IV. Justification of Refuge Configuration in MRID No. 455770-01. Additional research currently underway is summarized in MRID 453484-01 (Table 1). Pest biology is important to refuge placement since the goal is to encourage random mating between pests emerging from the transgenic and non-transgenic corn fields. By placing a non-Bt refuge in close proximity to a Bt corn field, insects that may carry an allele for resistance will potentially encounter and mate with insects that don't have the resistance allele. According to Monsanto, current information indicates that non-Bt refuges should be located within or adjacent to MON 863 corn fields to ensure random mating and dilution of the frequency of resistant alleles. Planting refuges adjacent to or within MON 863 fields is based on potential for and relative distance of adult dispersal.

Several factors will influence CRW fitness and survival including environmental influences, intraspecific and interspecific competition and later adult emergence. Earlier-emerging adults have been found to be more fecund and demonstrate a higher rate of survival than later-emerging adults (Boetel and Fuller 1997). Adult emergence may be delayed by planting later because corn hybrids grow more nodes as they mature which leads to more CRW adults per plant. Time of

flowering and temperature also influence when adults emerge (Bergman and Turpin 1984, Naranjo and Sawyer 1988, Elliot *et al.* 1989, Davis *et al.* 1996, Stavisky and Davis 1998). According to Monsanto, studies conducted thus far with MON 863 corn suggest that adult emergence is delayed seven to ten days when developing on Bt then non-Bt hybrids.

Adult WCRW and NCRW feeding and behavior studies were conducted in 1999 to compare silk clipping and within plant spatial distribution in Bt, non-Bt and insecticide treated plots (Appendix C). At the six locations tested, there were few significant differences observed in silk clipping, total number of CRW per plant or adult location within the plant canopy. These results suggest that MON 863 does not repel CRW adults.

According to Monsanto's submission, NCRW and WCRW adults are considered highly mobile insects. Male and female adults are known to fly at different heights within the corn canopy and wind has been shown to deter flight initiation of CRW. Since higher quality food is preferred by adults, CRW fly from more mature corn fields to younger fields, resulting in between field movement. Current unpublished research conducted by Dr. Joseph Spencer (University of Illinois) indicated that millions of male and female WCRW fly over and land in corn fields daily (MRID 453484-01 IRM Trial No. 13). Results of mark-and-recapture studies indicate that male and female adult WCRW will move within and between corn fields (MRID 453484-01 IRM Trial No. 13). NCRW demonstrate more limited movement than WCRW and are not known to migrate long distances. However, NCRW do migrate to new fields particularly if the habitat quality is improved which will ensure the colonization of new fields.

Adult female WCRW are generally believed to mate within the field they emerge from; whereas, adult males and mated females may move between fields. The significant dispersal observed in males can dilute resistance alleles present in a population. Based on the movement of adult CRW, planting refuges adjacent to or within fields will aid in promoting random mating. Experiments conducted by Monsanto suggest that virgin females may fly distances up to ten or more rows and are readily located by males when population densities are high.

Information is limited on CRW dispersal capabilities which is important in the development of an IRM strategy. Dr. Joseph Spencer will continue research on male and female dispersal prior to mating. This research will focus on determining the time males encounter newly emerged females and mark-and-recapture studies will be conducted to evaluate male dispersal capabilities. Additional studies will be conducted with flight mill assays to evaluate unmated female dispersal potential at Iowa State University by Ted Wilson. Another method of studying dispersal and mating behavior of WCRW is being developed by Dr. Doug Tallamy (University of Delaware). Dr. Tallamy is breeding a colony with red elytra that will have potential use in field research. WCRW in Illinois, Indiana and Ontario will also be examined by Dr. Art Schaafsma (Ridgetown College/University of Guelph) for genetic variation between variant and nonvariant populations. This research will provide information on gene flow that can be considered when designing an IRM strategy.

Reviewer's Comments

Monsanto has identified information on CRW biology from published articles as well as current

research currently underway that is important to develop a long-term IRM strategy for CRW in MON 863 corn. Additional information is needed on various aspects of CRW pest biology as it relates to an IRM strategy. Knowledge of the maximum and average distance adult and larval CRW move is limited. Additional research regarding male and female (mated and unmated) adult and larval WCRW and NCRW dispersal potential is needed to determine placement of non-Bt corn refuges. In addition, more information is needed on mating habits, ovipositional patterns, number of times a female can mate and fecundity.

There are three *Diabrotica* species found in corn, WCRW (*Diabrotica virgifera virgifera*), NCRW (*Diabrotica barberi*), Mexican corn rootworm (MCRW; *Diabrotica virgifera zea*) and the southern corn rootworm (SCRW; *Diabrotica undecimpunctata howardi*). Knowledge of CRW biology, dispersal characteristics, host range, feeding habits and history of insecticide resistance is important in developing an IRM strategy. The WCRW and NCRW are the most serious insect pests of field corn in the Corn Belt (Levine and Oloumi-Sadeghi 1991). The SCRW (also known as the spotted cucumber beetle) is a polyphagous pest with over 280 host plant species and predominantly feeds on cucurbits (http://ipmwww.ncsu.edu/AG271/corn_sorghum/southern_corn_rootworm.html). The SCRW does not cause serious injury to corn roots in the Corn Belt because of their late arrival. In the U.S., the MCRW is known to occur in Kansas, Oklahoma, and Texas (Levine and Oloumi-Sadeghi 1991). A study conducted by Branson *et al.* (1982) showed no differences in developmental rates of WCRW and MCRW. However, additional studies have shown that the MCRW larvae take longer to develop than WCRW (Jackson and Elliott 1988, Woodson and Chandler 2000). Additional research is needed to determine if IRM strategies designed for WCRW and NCRW are appropriate for MCRW. Since MON 863 expression in corn roots likely declines as the plant matures and MCRW may develop slower the risk of resistance may differ for MCRW than WCRW and NCRW.

Characteristics of pest biology that are relevant to IRM (e.g., movement, feeding habits and ovipositional habits) differ for WCRW and NCRW. WCRW and NCRW adults will feed on corn silks, pollen and young kernels in the ear tip; however, only WCRW feed on leaves. Since NCRW adults don't feed on corn leaves, they leave the field after pollination to find a younger field with pollen available (Branson and Krysan 1981). Young leaves contain the highest level of MON 863 with an average of 81 µg/g, grain has an average of 70 µg/g, root and forage have an average of 39 and 41 µg/g, pollen has an average of 62 µg/g and silks contain the lowest levels with an average of 10 µg/g (MRID No. 451568-02). Since adult and larval CRW feed on various parts of the corn plant, both life stages may be exposed to the Bt protein and extended selection pressure may result (Meinke *et al.* 2001). Severe root damage from larval feeding will lead to plant lodging and yield losses.

WCRW and NCRW are univoltine, develop through one generation per year, in corn in most of the Corn Belt (Branson and Krysan 1981, Meinke *et al.* 2001). CRW typically oviposit where the adults are feeding which is almost exclusively in corn fields (Branson and Krysan 1981, Levine and Oloumi-Sadeghi 1991). In general, CRW adult emergence varies based on species, geography, weather, management practices such as insecticide use, population density and sex. For instance, males typically emerge before females and emergence, as well as fecundity, longevity and egg viability, are reduced in corn planted later in the season (Boetel and Fuller

1997, Levine and Oloumi-Sadeghi 1991, Meinke *et al.* 2001). It is unknown what effect corn rootworm protected transgenic corn will have on phenology, sex ratio and adult emergence patterns. According to the NCR-46 (a technical committee consisting of research and extension CRW specialists and other cooperators), adult CRW emergence is prolonged four to six weeks in MON 863 corn then non-transgenic corn (Meinke *et al.* 2001). Asynchronous adult emergence for Bt corn fields and non-Bt refuges may lead to nonrandom or assortive mating which may lead to an increase rate in the evolution of resistance. Nonrandom or assortive mating may also occur if Bt corn disrupts the synchrony of male and female CRW adult emergence (Meinke *et al.* 2001). Mating typically occurs within 24 to 48 hours of female adult emergence within the corn fields they emerged from or nearby (Meinke *et al.* 2001).

CRW larval movement is limited particularly in areas with low population densities (Meinke *et al.* 2001). Published and unpublished articles have reported varying distances that CRW larvae move. WCRW larvae may move from 12 to 16 inches and have been found in corn rows planted up to 40 inches apart (Suttle *et al.* 1967, Short and Luedtke 1970, Gray 1999). These studies suggest that CRW larvae hatching from eggs between rows are capable of finding and injuring corn roots regardless of row spacing. Since field corn is typically planted approximately 24 to 30 inches apart, CRW may move between two to three rows. In general, young CRW larvae (e.g., 1st, 2nd and sometimes 3rd instars) tend to move toward actively growing corn roots. Larval tendency toward respiring, growing corn roots is probably because of their ability to detect and move toward CO₂ (Strnad *et al.* 1986, Gray 1999). Young larvae will feed on the distal portion of corn roots and move through the soil to feed on new, short roots as they develop into later instars (Strnad and Bergman 1987, Gray 1999). It is therefore possible that a RS heterozygous larvae with a partially recessive resistance trait will begin feeding on transgenic corn roots and finish its development on adjacent non-transgenic roots which would result in a non-lethal dose of MON 863 and potential resistance. Movement of larvae is deterred by soil that is too wet or too dry and is greater in silty clay or loamy soils than loamy sand soils (Gray 1999).

NCRW and WCRW mated adults may be very mobile and have potentially high dispersal capabilities (Meinke *et al.* 2001). However, local dispersal is more common and involves movement within or among adjacent fields; whereas, migratory dispersal over long distances occurs in a small portion of populations and usually involves females (Meinke *et al.* 2001). Research conducted by Van Woerkom *et al.* (1983) showed that CRW are typically low fliers, wind affects beetle mobility and deters flight initiation, females fly higher than males and are found in greater numbers above the crop canopy. Dispersal capabilities of the WCRW are greater than the NCRW. The WCRW is also a greater competitor and displaced the NCRW in Nebraska by 1980 (Hill and Mayo 1980). WCRW post-mating dispersal may be local or migratory.

Coates *et al.* (1986) used mated WCRW females collected from the field in a flight mill to determine duration and length of flights. Trivial flights (<30 min.) lasted from one to 17 minutes and sustained flights (≥30 min.) for females less than nine days old occurred up to four hours. Based on this data, Coates *et al.* (1986) speculated that preovipositional WCRW females capable of unassisted flights could move an average of 35 km per day (200 km overall) for the first six days after emergence. This data suggests that some females may leave the field after mating to oviposit elsewhere. While sustained flights by mated female CRW are possible, movement by

unmated females is limited. Knowledge of the maximum and average distance an adult CRW moves is limited. Additional research regarding adult and larval WCRW and NCRW dispersal potential is needed to determine placement of non-Bt corn refuges.

CRW Adaptive Strategies:

Summary of Monsanto's Proposal

CRW have developed several adaptations to control methods including crop rotation and insecticide resistance. A common agronomic practice used to control CRW involves rotating corn with soybeans. Rotating soybeans after corn decreases the need for CRW-targeted insecticide applications. However, WCRW has developed an adaptation to resist the corn/soybean rotation in Illinois and Indiana (Levine and Oloumi-Sadeghi (1996). WCRW have been found to lay their eggs and overwinter in soybeans allowing them to emerge in corn fields the following spring. NCRW populations have also developed resistance to the corn/soybean rotation in Minnesota, Iowa, and South Dakota (Gray *et al.* 1998). NCRW have developed the ability for extended diapause resulting in a significant proportion of their eggs hatching after two winters.

The WCRW has also developed resistance to soil-applied organochlorines as well as organophosphate and carbamate sprays in Nebraska (Ball and Weekman 1963, Metcalf 1983). Organochlorine insecticides were used as pre-plant treatments in the 1940's and resistance occurred by the 1950's. Cyclodiene insecticides including aldrin, chlordane and heptachlor were used in the 1950's to control root feeding damage; resistance to these chemicals was observed after 12 years and led to widespread control failures within 20 years. Organophosphate and Carbamate insecticides were introduced to replace Cyclodienes. However, Meinke *et al.* (1998) found adult populations resistant to organophosphates and carbamates in Nebraska and also documented control failures of methyl parathion in the early 1990s.

Instances of CRW resistance to crop rotation and/or insecticide use typically develop on a local scale which is probably due to limited adult movement before and after mating. In these cases, resistance took at least ten and usually more than 15 years to develop with out implementing IRM strategies. Research is currently underway at the University of Nebraska and USDA-ARS in North Dakota to determine the genetics of esterase-mediated insecticide resistance in WCRW populations. Results of this research are intended to provide knowledge on localized selection and migration that may aid in refining future IRM strategies.

Reviewer's Comments

WCRW has developed resistance to certain pesticides and crop rotation practices. For instance, WCRW was four times less susceptible to aldrin in 1981 than when it began to be used 20 years earlier in Nebraska and most corn acres were affected by 1980 (Ball 1983, Metcalf 1986, Gray 1999). Additional WCRW resistance to methyl parathion and carbaryl was documented by Meinke *et al.* (1998) in Nebraska and heritability of the resistant trait was shown from F₁ generation individuals. A 16.4-fold resistance to methyl parathion and 9.4-fold increase in resistance to carbaryl was determined (Meinke *et al.* 1998). There has been some research

conducted to determine the mechanism of insecticide resistance in CRW populations. Studies were conducted by Miota *et al.* (1998) to examine two WCRW populations to determine the mechanism of methyl parathion and ethyl parathion. Resistance was due to a combination of metabolic detoxification and target site insensitivity; however, the mechanism of resistance differed among the observed populations. Miota *et al.* (1998) concluded that the mechanism of resistance for one of the observed populations was due to increased metabolic detoxification by NADPH-dependent monooxygenases and general esterases and another population was due to and interaction of acetylcholinesterase sensitivity and hydrolytic metabolism.

It is likely that the mechanism of CRW resistance differs between insecticides and Cry3Bb1. The mechanism and genetics (e.g., one gene, multiple genes) of potential resistance of CRW to MON 863 needs to be determined to develop an appropriate IRM strategy. Since CRW resistance to Cry3Bb1 under field conditions is necessary to determine the mechanism and genetics of resistance, colonies resistant to Bt should be established and evaluated in the laboratory during the initial three years MON 863 is grown commercially.

Since CRW predominantly oviposit in corn fields, rotating corn with small grains, hay, clover or alfalfa has been utilized as a control method (Levine and Oloumi-Sadeghi 1991). CRW have also been controlled by planting soybean after corn since CRW cannot survive on soybean. However, in areas such as east-central Illinois and northern Indiana, the WCRW has been found to have the ability to lay eggs in soybean, overwinter and hatch the following year in corn (Levine and Oloumi-Sadeghi 1991, Levine and Oloumi-Sadeghi 1996, O'Neal *et al.* 1999, Isard *et al.* 1999, Isard *et al.* 2000). NCRW has developed a prolonged or extended diapause adaptation to rotating corn with crops such as soybean. Prolonged diapause of NCRW involves eggs that remain viable for two winters and hatch two seasons after being laid. Extended diapause has been verified in the laboratory from NCRW eggs collected from South Dakota, Minnesota, Illinois and Michigan (Krysan *et al.* 1984, Krysan *et al.* 1986, Levine and Oloumi-Sadeghi 1991, Levine *et al.* 1992a, Levine *et al.* 1992b). Field studies conducted by Tollefson (1988) in northwestern Iowa corn fields suggests that extended diapause occurs throughout NCRW distribution in rotated fields. Another study conducted by Levine and Oloumi-Sadeghi (1996) suggests that the WCRW does not demonstrate extended diapause. The effect of WCRW ovipositing in soybean prior to overwintering and extended diapause in NCRW on an IRM strategy needs further investigation.

Dose:

Summary of Monsanto's Proposal

Determining the level of dose is crucial to what size and structure of a refuge is needed to delay CRW resistance to Cry3Bb proteins. Monsanto identifies a high dose and low dose strategy for delaying the development of resistance. A high dose strategy would involve killing a high proportion of the target pest population. As part of the IRM strategy, the high dose would be used in conjunction with a structured refuge designed to produce a large number of susceptible pests. The high dose/refuge strategy is currently in use to delay European corn borer (ECB; *Ostrinia nubilalis*) resistance to lepidopteran-active Bt crops.

A low dose strategy identified by Monsanto is employed for products that have limited impact on the fitness, survival and selection pressure of the target pest. Products with a low dose may not require a refuge and may have a longer period of durability. In general, refuge size for a low dose product is not well understood. With a low dose strategy it may be difficult to sufficiently control the pest while limiting effect on its fitness. Since CRW feed on corn roots and some level of this damage may be tolerated by the plant, Monsanto proposes that a low dose strategy may be appropriate.

According to Monsanto, research on CRW feeding behavior and survival and root expression data will help identify the dose of MON 863. Studies recently conducted by Monsanto as well as Dr. John Foster and Mr. Pete Clark (University of Nebraska - Lincoln) show that CRW larvae that feed on MON 863 corn begin feeding on growing root tips, but don't penetrate into the root (Appendix C). CRW larvae feeding on corn roots were observed to graze around the exterior of the root without penetrating, thus slowing their development. Larval feeding behavior observed on MON 863 corn roots may be due to an adverse reaction of CRW taste receptors or a physiological effect leading to a change in feeding behavior. A number of roots evaluated in the field by Root Damage Rating (RDR) had channeling on the outside of roots and no penetration. The RDR is based on a 1-6 rating scale and is considered the most sensitive and consistent method of evaluating CRW damage (Branson *et al.* 1980, Knutson *et al.* 1999). Lower values on the root rating scale are considered to have a lower level of damage. A reduction in CRW feeding on MON 863 was demonstrated by a screening trial conducted by Monsanto that showed an average damage rating for MON 863 to be 1.85 on the RDR scale compared to a non-Bt check which had a 4.44 RDR rating (MRID 451568-05, Table 3 on pg 61).

The dose level of MON 863 against WCRW larvae was determined by root expression assays and CRW bioassays conducted by Dudin *et al.* (2001). Results of this study determined the Cry3Bb1 expression in corn roots is 58 ppm which is slightly lower than the LC_{50} of 75 ppm determined from laboratory colonies (see Monitoring section for LC_{50} information). Cry3Bb1 protein expression is thought to be high in the root tips where neonate larvae initiate feeding. Neonates that avoid toxic doses of MON 863 because of the grazing behavior observed on the exterior of roots may tolerate the Bt toxin during the remainder of their development. Therefore, Monsanto concluded that a high dose strategy is not appropriate for MON 863 expressing corn based on the LC_{50} determined from root feeding and a reduction in susceptibility of second and third instar larvae observed in laboratory bioassays. Monsanto also suggests that efficacy studies indicate that MON 863 provides improved and more consistent protection of corn roots than insecticides (MRID 453613-03). In corn with the same CRW larval pressure, an average root rating of 2 has been determined for MON 863, whereas an average root rating of 3 has been determined for insecticide treated corn. This indicates a higher level of protection provided by MON 863 than currently registered larval insecticides.

A high dose can be defined as <0.001% survival of larvae (FIFRA SAP 1998); therefore, larval survival is another technique that may be utilized to determine dose. Survival of CRW larvae on MON 863 expressing roots has been estimated to range from 17% to 62% (Appendix C). Gray *et al.* (1992) found insecticide control of CRW larvae is highly variable. This variability may be due to a lack of root availability leading to density-dependent mortality, soil moisture and rainfall, characteristics of insecticides, application rate and methods (Branson and Sutter 1985,

Elliott *et al.* 1989, Levine and Oloumi-Sadeghi 1991). These same variables that affect efficacy of insecticides will likely affect effectiveness of MON 863.

Data from additional research being conducted will help identify MON 863 dose and effective refuge size. Dr. Lance Meinke (University of Nebraska) is studying fitness of WCRW feeding on MON 863 including fecundity, longevity, developmental time, size and sexual competitiveness of males feeding on MON 863 root tissue in the greenhouse.

Reviewer's Comments

For lepidopteran-active Bt corn products, a high dose is recommended to delay resistance in ECB. A high dose for lepidopteran-active Bt proteins is defined as 25 times the amount of Bt delta-endotoxin necessary to kill susceptible individuals (SAP 1998). Bt plant incorporated protectants (PIPs) could be considered to provide a high dose for ECB if verified by at least two of the following five approaches: 1) Serial dilution bioassay with artificial diet containing lyophilized tissues of *Bt* plants using tissues from non-*Bt* plants as controls; 2) Bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar determined by quantitative ELISA or some more reliable technique; 3) Survey large numbers of commercial plants in the field to make sure that the cultivar is at the LD_{99.9} or higher to assure that 95% of heterozygotes would be killed (see Andow and Hutchison, 1998); 4) Similar to #3 above, but would use controlled infestation with a laboratory strain of the pest that had an LD₅₀ value similar to field strains; and 5) Determine if a later larval instar of the targeted pest could be found with an LD₅₀ that was about 25-fold higher than that of the neonate larvae. If so, the stage could be tested on the *Bt* crop plants to determine if 95% or more of the later stage larvae were killed (SAP 1998). It has not been determined if this definition and verification of a high dose is appropriate for coleopteran-active Bt corn hybrids nor has it been concluded that a high dose is necessary to mitigate resistance.

For a high dose strategy, a resistance (*R*) allele frequency should be <.001 (Andow and Alstad 2002). According to Monsanto, a high dose strategy for coleopteran protected corn would involve killing a high proportion of the target pest population. However, according to the definition of a high dose (SAP 1998) all susceptible insects would be killed. Monsanto provided data that suggest 17-62% of CRW larvae may survive when feeding on MON 863. Therefore, it can be concluded that MON 863 does not have a high dose for control of CRW.

A moderate dose was defined by Monsanto in their use of Caprio's model as 30% survival of larvae and a low dose as 50% survival. Since data suggests that there is >17% survival of CRW larvae feeding on MON 863 corn roots, it can be concluded from the modified Caprio model that there is a low to moderate dose of MON 863 for CRW.

"Further research is needed to provide the critical data on the actual dose effects of Bt corn, corn rootworm biology and ecology" (Ostlie, 2001). Ongoing research is being conducted by Monsanto. Although adult CRW will feed on corn tissue expressing the Cry 3Bb1 protein, larval feeding on corn roots is the predominant mode of exposure. Therefore, expression of MON 863 in roots is particularly important to determining the level of MON 863 CRW are exposed to. First and second instar WCRW larvae have been observed to feed on the meristematic region of

the root or tip (MRID No. 455770-01 Appendix C). Less older instars have been found on MON 863 roots than non-transgenic corn roots when feeding during the V4 and V6 growth stages; however, older instars did not seem to be affected by the V8 growth stage (MRID No. 455770-01 Appendix D). Preliminary results of Monsanto's root expression study can be found on pg. 99, Figure 4a-h of MRID No. 455770-01 and an explanation of acronyms is found on pg. 95. A final report of these studies should be submitted to the Agency for review upon completion. Research is being conducted in Wharlon, TX to evaluate the efficacy of transgenic coleopteran-protected Bt corn against MCRW (http://usda-apmru.tamu.edu/WCH/evaluation_of_genetically_modifiedi.htm).

Simulation Models of Resistance:

Summary of Monsanto's Proposal

Simulation models are used to evaluate the importance of IRM and appropriate strategies to delay resistance. Assumptions in resistance models are based on aspects of pest biology including CRW survival and fitness. A conservative model developed by Dr. Michael Caprio (Mississippi State University) was modified and used by Monsanto to predict the risk of CRW developing resistance to MON 863 corn (Tables 2 and 3). Caprio's model assumes 100% market penetration, no alternate host crops, no fitness costs in resistant CRW and that resistance is due to a single gene. Resistance due to multiple genes would probably occur slower or at the same rate as single gene influences. Based on results of this model, Monsanto concluded that a 20% non-Bt refuge planted within or adjacent to MON 863 fields would delay resistance for 11 years regardless of dose. Since there will not be a 100% initial adoption of MON 863 corn, Monsanto suggests that the "effective refuge" will be much greater than 20%.

Two models, one developed by Dr. David Andow (University of Minnesota) and another developed by Dr. David Onstad and Mr. Charles Guse (University of Illinois), will also be used by Monsanto to develop their long-term IRM plan (Table 4). The Onstad and Guse model considers four crops (continuous corn, first year corn, soybean and a non-corn or cucurbitaceous crop) and six fields (up to four corn fields). Their model suggest that planting refuges as blocks would delay CRW resistance longer than in-field strips (Onstad *et al.* 2001). According to Monsanto, Onstad and Guse's model as well as the Monsanto modified Caprio model indicate that product durability is not greatly affected by refuge size in low and moderate dose CRW protected Bt corn. See Figure 1 on page 26 of MRID 455770-01 for a graphic comparison of refuge size and dose on the number of years to development of resistance shown by Caprio's model. Andow's model considers typical midwestern planting practices including continuous and rotated Bt or non-Bt corn and soybean. Therefore, Andow's model (as well as Onstad's model) accounts for CRW populations ability to adapt to crop rotation.

Assumptions of models created thus far have not been tested or validated. Field testing and validation of these models will be continued during the three year interim period requested by Monsanto. Additional models will also consider the potential of seed mixtures, dose and behavioral avoidance.

Table 2. Input values for MON 863 performance parameters used in Caprio simulation.

These values are applicable for low to moderate dose product.

Parameter	Input Value
Survival of SS genotype	0.1 - 0.5
Survival of SR genotype	1
Survival of RR genotype	1
Refuge size (%)	0 - 60
Initial resistance allele frequency, q ($t=0$)	0.001
Move outside habitat before mating (R)	1
Move outside habitat before ovipositing (M)	1

Table copied from MRID 455770-01 page 25 Table 3.

Table 3. Caprio model predictions of product durability, expressed in years to resistance as a function of refuge size and dose (*i.e.*, survival rate). Input values for the low and SS genotype was set at 0.01 and survival of the SR genotype was set at 0.1.

Survival Rate	Refuge Size (%)			
	0	20	40	60
0.01 (high dose)	4	19	42	86
0.3 (moderate dose)	8	11	16	26
0.5 (low dose)	13	17	24	27

Table copied from MRID 455770-01 page 25 Table 4.

Table 4. Monsanto's comparison of model structure, parameters, and assumptions for Andow and Onstad's models

Parameter or Description	Andow Model	Illinois Models
Model type	stochastic	stochastic
Model structure	5-patch w/populations in +/- CRW cont. corn, +/- CRW rotated corn, soybean	4 to 6 patch w/populations in +/- CRW cont. corn, +/- CRW rotated corn, soybean, & non-crop
Proportion of crop types	variable, tested 100% & 40% continuous corn	variable, tested 100% continuous corn
Time Step	yearly	daily, day 187-292
Overwintering egg survival	combined with fecundity, default: 50 viable overwintering eggs/female	50%
Density-dependent larval survival	Hassel function w/parameters fit to survival data from artificial infestations or field sampling	exponential function fit to combined data from artificial and field sampling
Larval survival on transgenics	variable (0.001-0.5), imposed prior to density dependent mortality	variable (0.001-0.5), imposed prior to density-dependent mortality

Parameter or Description	Andow Model	Illinois Models
Beetle emergence	–	37 day period, mean emergence: day 207 - males, day 215 - females
Mating pattern	females mate 1 time	females mate up to 2 times, males mate once every 2 days and search 100 m ² per day
Premating dispersal	random in field, no between field movement	teneral females do not move, random within field
Post mating dispersal	proportional to patch area	mated females distributed proportional to patch size, between fields: 0.02 mated females/day, 0.005 males/day
Oviposition/fecundity	distributed proportional to crop area and crop preference, 50 overwintering eggs/female	random within field, oviposition period: 60 (1 st) & 40 (2 nd) days, 440 eggs/female, viability declines with beetle age
Beetle survival	–	influenced by frost (day 292), toxicity of transgenic to adults, nutritional status of other crops, and beetle age
CRW Genotypes	SS, SR, RR	SS, SR, RR
Dominance	variable (0.05-1)	variable (0, 0.5, 1)
Fitness cost of R genes	none	none
Initial resistance gene frequency	variable (10^{-3} to 10^{-5})	variable (default 10^{-4})
Definition of resistance	gene frequency >0.5	gene frequency >0.3
Refuge placement	separate fields	separate fields that remain in same location over time or row strips within field
Treatment of refuge	no	default: no

This table is copied from page 26 Table 1 of MRID 451568-05

Reviewer's Comments

EPA has used predictive models to compare IRM strategies for Bt crops. Because models cannot be validated without actual field resistance and other data, models have limitations and the information gained from the use of models is only a part of the weight of evidence used by EPA in assessing the risks of resistance development. It was the consensus of the 2000 FIFRA SAP Subpanel that models were an important tool in determining appropriate Bt crop IRM strategies. They agreed that models were “the only scientifically rigorous way to integrate all of the biological information available, and that without these models, the Agency would have little scientific basis for choosing among alternative resistance management options.” They also recommended that models must have an agreed upon time frame for resistance protection. For

example, conventional growers may desire a maximum planning horizon of five years, while organic growers may desire an indefinite planning horizon. The Subpanel recommended that model design should be peer reviewed and parameters validated. Models should also include such factors as level of Bt crop adoption, level of compliance, economics, fitness costs of resistance, alternate hosts, spatial components, stochasticity, and pest population dynamics.

Simulation models are an important tool used to predict possible strategies (e.g., refuge size and structure) to delay insect resistance. Knowledge of pest biology is necessary to creating useful models. Since CRW adults and larvae feed on corn tissue and MON 863 is expressed throughout the plant, models should consider the exposure to all life stages (Gray 1999). Information is still needed to develop appropriate simulation models of resistance for CRW-protected corn. For instance, the initial resistance (*R*) allele frequency in CRW for Cry3Bb proteins is unknown.

A limited number of models have been developed to address IRM strategies for CRW protected corn. Monsanto modified a model developed by Caprio (1998) for cotton bollworm (*Helicoverpa zea*; CBW) to address CRW biology and IRM. Additional models developed for WCRW by Andow and Alstad (2002) and Onstad *et al.* (2001) are summarized below. These models assume 100% grower adoption of Bt corn.

Caprio Model (ILSI-HESI 1998)

A conservative model originally developed by Dr. Michael Caprio (Mississippi State University) for cotton bollworm (*Helicoverpa zea*; CBW) resistance in cotton was modified by Monsanto to predict the risk of CRW developing resistance to MON 863 corn. MON 863 performance parameters were used in Caprio's deterministic nonrandom mating model described in the International Life Sciences Institute Health and Environmental Sciences Institute (ILSI-HESI) report (1998). This model was designed to run with sprayed and unsprayed refuge options. Initially, the entire population in this model is divided into two habitats including transgenic fields and refuge fields. The population is subjected to user-defined parameters of selection pressure to determine what level of the surviving population is SS (homozygous susceptible), SR (heterozygous), and RR (homozygous resistant) genotypes. Input values used by Monsanto for low and moderate dose included: 1) Survival of SS genotype = 0.1 - 0.5 ; 2) Survival of SR genotype = 1; 3) Survival of RR genotype = 1. A high dose simulation of survival was run with input values of 0.01 for the SS genotype and 0.1 for the SR genotype. However, it has not been determined if these values are appropriate for a simulation model addressing CRW resistance management.

Application of non-selective insecticides to refuges is also considered. Insecticide application is assumed to reduce the population size without changing relative allele frequencies. There is a portion of the population assumed to disperse prior to mating and a part that mates before dispersing resulting in random and non-random mating patterns (ILSI-HESI 1998). Monsanto used an input value of 1 to identify the portion that move outside the habitat before mating (*R*) and the portion that move outside the habitat before ovipositing (*M*). However, female CRW typically mate within the field they emerge from. Since females do not leave the field prior to

mating, an input value of 1 for R is probably inappropriate for CRW IRM models. Postmating, preoviposition dispersal is assumed in this model and individuals that mate randomly will oviposit at random and these eggs are divided between transgenic fields and refuges. Eggs produced from non-random mating are assumed to be laid in the field (Bt or non-Bt) where mating occurred (ILSI-HESI 1998). However, it is likely that mated CRW females leave the field to oviposit so this parameter of the model may not apply to MON 863. The resistance allele frequency Monsanto used in this model was 0.001 and a 0 - 60% refuge size was considered.

According to the results of this model, a 20% refuge for a high dose product (0.01 survival rate) would delay resistance for 19 years, a moderate dose (0.3 survival rate) would delay resistance for 11 years, and a low dose product (0.5 survival rate) would delay resistance for 17 years. These results suggest that a 20% refuge in a low dose product would delay resistance 30% longer (17 years vs 13 years) than no refuge and 27% (11 years vs 8 years) for a moderate dose product. However, further investigation into the validity of this model for the CRW is needed. Assuming MON 863 confers a low to moderate dose, delaying resistance for 11 years may not be an adequate duration. This model also does not use a stochastic simulation nor does it consider spatial factors involved in the evolution of resistance. Further refinement of this model is needed.

Andow and Alstad Model (Andow and Alstad 2002)

Drs. David Andow and David Alstad (University of Minnesota) developed a deterministic model for resistance evolution of WCRW using between field refuges. This patch model addresses WCRW ability to oviposit in soybeans or corn and accounts for a corn-soybean or corn-alfalfa cropping system. Areas with 100% continuous corn and areas with 40% continuous corn and 60% crop rotation for a total of 70% potential corn acreage (40% continuous + (60/2)% rotated) are considered in this model. WCRW ability to oviposit in soybeans at the end of the season and emerge in corn fields the following spring is included in this model. A 5-50% refuge is assumed for continuous and rotated corn and refuges are planted in alternating blocks with transgenic corn. High risk areas with considerable levels of damage to first year corn are addressed in this model. Based on CRW biology, this model assumes pre-mating dispersal is negligible, random mating within fields and a high rate of post mating dispersal which is appropriate based on CRW biology. Five types of patches are included and post mating dispersal among patches is random. It is assumed that there is no pest management on the refuges, however, application of soil applied insecticides are expected to be used on refuge acres. According to Andow, the fundamental questions addressed in this model are “(a) how does the rate of evolution of resistance depend on the relative areas of the different crops, especially the proportion of non-transgenic refuge, and (b) how sensitive are these rates to variation in the many parameters. Specifically, we evaluate the effects of SS survival (μ), dominance (O), initial R allele frequency (p_0), fecundity (fec), preference for ovipositing on soybean (D), and density dependence ($\%$ and $\$$).”

There was no difference in the number of WCRW generations needed to exceed a 0.5 resistance (R) allele frequency for continuous versus rotated corn (40% continuous) with a 20% refuge in first year and continuous corn, when oviposition is allowed in soybean and $p_0=0.00001$ (a very

low resistance allele frequency). Results of this model indicated that there was virtually no difference between 100% continuous corn and 40% continuous corn simulations in the number of generations needed for the *R* allele frequency to exceed 0.5. Survival of susceptible homozygotes (*SS*) and heterozygote dominance was varied in the model over a wide range. Results indicated that susceptible homozygotes would remain susceptible to transgenic corn for < 15 generations. This is probably because local mating is leading to assortive mating among dominant resistant (*RR*) genotypes. If *SS* survival is large enough and dominance is small enough, then > 15 generations may occur before 0.5 *R* allele frequency is reached and a low dose IRM strategy may be effective. A high dose strategy is only effective under a narrow range of parameter values (*SS* survival <0.01 and dominance <0.05).

Another set of scenarios (e.g., 40% continuous corn, 20% refuge, potential oviposition in soybean, varied survival of homozygotes and heterozygous dominance) examined the effect of the initial *R* allele frequency on resistance management strategies. The initial *R* resistance allele frequencies varied from 0.00001 (best case scenario), to 0.0001 and 0.001 (worst case scenario). Results of this model predict that resistance could not be delayed for > 15 years with a high dose strategy for WCRW and a 20% refuge planted between Bt fields when *SS* survival was less than or equal to 0.02. Simulations suggest that resistance may be delayed for > 15 years with a low dose strategy if the *SS* survival is \$0.2 and heterozygote dominance is #0.17. If MON 863 only produces a low dose rather than a moderate dose for control of WCRW, this model predicts that resistance may be delayed > 15 years in areas of high adoption of continuous corn.

This model was also run to evaluate the sensitivity of fecundity (varied from 20-220 viable eggs/female) and percent refuge (varied from 5-50%) in 40% continuous corn, $p_0=0.00001$, oviposition in soybean allowed, and susceptible homozygote survival and heterozygote dominance fixed for a high or low dose. Results of this run indicated that fecundity and variation in refuge size did not affect durability of resistance for high or low dose simulations. The lack of effect refuge has on the evolution of resistance is probably due to local mating which results in reduced dilution on homozygous recessive genotypes and susceptible genotypes.

Another simulation was run that considered a preference to oviposit in soybean, a 5-50% refuge, 40% continuous corn, $p_0=0.00001$, fecundity of 50 viable eggs/female and susceptible homozygote survival and heterozygote dominance fixed for a high or low dose. Risk of resistance was not affected by a preference for WCRW to oviposit in soybean versus corn which implies that ovipositional preference is probably not affecting the outcome of these models nor durability of resistance.

A simulation was also run to evaluate variance in density dependent values and percent refuge (5-50%), using parameter values of 40% continuous corn, $p_0=0.00001$, oviposition in soybean allowed and susceptible homozygote survival and heterozygote dominance fixed for a high or low dose. Density dependent factors considered were larval density at which strong density dependence occurs (%) and the level at which density dependent mortality intensifies with increasing density (\$). There is uncertainty in the estimation of % and \$.

Results of the simulations indicate that these parameters probably do not affect the durability of resistance nor conclusions based on the model. Andow and Alstad's model suggest that between

field refuges for resistance management of high dose ($\mu=0.01$, $\sigma=0.05$) transgenic CRW protected Bt corn are likely to be ineffective. However, a low dose ($\mu=0.2$, $\sigma=0.2$) strategy with a 20% between field refuge would probably delay resistance for >15 generations because selection pressure in favor of resistance is weak. Details of this model can be found in MRID 455770-01 Appendix H. Further refinement of this model is needed.

Onstad Model (Onstad *et al.* 2001)

Onstad's patch model is based on the biology, population dynamics and genetics of the WCRW and was designed to address corn (continuous or rotated), soybean and other crops (e.g., oats or alfalfa). This model assumes resistance is due to two loci and two alleles per locus because resistance to both crop rotation and transgenic corn are considered. It also allows for the possibility of incorporating the use of soil insecticides or seed treatments and assumes the refuge is maintained in the same place each year. An initial R allele frequency is assumed to be 0.0001 in continuous corn and the simulation measured the time to reach $R=0.03$.

Allele expression in the CRW and dose were the two most important factors affecting the outcome of the model. A dominant resistance allele led to the quick evolution of resistance to CRW protected Bt corn; whereas, a recessive allele delayed resistance for more than 99 years. In other words, when resistance is recessive, the resistance allele frequency did not exceed 3% for the 99-year period simulated. However, when resistance is dominant, the resistance allele frequency rapidly exceeded 3% within two to nine years as refuge size ranged from 5 to 30% for all doses of the toxin greater than 0.001.

Genotype, field and age are distinguished for adult males and unmated females; whereas, mated females are distinguished by genotype, field, age and genotype of mate. Aspects of corn phenology (period between anthesis to maturation is considered) and pest biology including phenology adult dispersal, sexual activity, oviposition, sex ratio and survival of immature beetles are considered in this model. However, it is unclear why this model considers "remating" of female.

Results of this model account for delayed emergence by susceptible adult CRW and refuge structure such as planting as row strips or blocks. Row strips were shown to reach a resistance allele frequency of 0.3 much more quickly than a block configuration. These results showed that high dose products will delay resistance (time required to reach 0.03 resistance allele frequency) for 13 years to more than 99 years when the refuge size is varied from 5 to 30%. A block refuge size was not affected when a high dose (SS survival $\neq 0.001$) was considered as a 0.03% resistance allele frequency was not reached in > 99 years. For a high dose product, a 5% refuge planted as strips reached a 0.03 resistance allele frequency in 13 years and a 30% refuge will delay resistance for 34 years. A lower dose product (SS survival $\neq 0.05$ and $\neq 0.2$) with 5-30% block refuge was shown to delay resistance (time to a 0.03 allele frequency) for five to nine years respectively.

Lower dose products with a 5-30% refuge planted as row strips delayed resistance two to six years respectively. Resistance evolved faster in row strips than in a between field block refuge. Onstad *et al* (2001) indicate that the faster evolution of resistance is due to greater selection

pressure with row strips because of the greater likelihood of oviposition in the transgenic strips compared with separate blocks. Sensitivity analyses indicated that changes in parameter values had little effect on the time to resistance when resistance genes A and B are additive and a block configuration is used.

Onstad *et al.* (2001) simulate dose of toxin expression in the plant measured as the survival of susceptibles at 0, 0.001, 0.05, 0.1 and 0.2. MON 863 appears to have a low to moderate dose because data show 17% to 62% larval survival. What the dose MON 863 is actually producing is critical to the assumptions of this model (or any genetic model). If lower doses > 0.2 (survival of susceptibles) were used in the model, it is likely that the predicted years to resistance ($R=0.3$) would increase to greater than 10 years. This conclusion is the same whether the refuge is in-field strips or between field blocks. Further refinement of this model is needed.

Refuge:

Summary of Monsanto's Proposal

According to Monsanto's submission, a refuge will be required to be planted with MON 863 corn hybrids that will produce CRW susceptible to the Cry3Bb1 protein. Refuges will be allowed to be planted as continuous blocks adjacent to the MON 863 fields, perimeter strips or non-transgenic strips planted within transgenic fields. Since field surveys conducted in 1999 showed that adult CRW are not repelled by MON 863 (Appendix C), planting a refuge within or adjacent to the transgenic field will not deter adults from migrating into the refuge. Seed mixtures of transgenic and non-transgenic corn will not be allowed until information on its adequacy is gathered.

Growers will be required to plant one acre of non-Bt corn for every four acres of MON 863 corn planted. This is equivalent to planting a 20% non-Bt corn refuge and is based on the current understanding of CRW biology, Cry3Bb1 effective dose, preliminary modeling results and agronomic considerations. Monsanto expects a "*de facto*" refuge to exist on every farm because a limited availability of MON 863 hybrids are expected during the first three years of the commercial registration. Seed and granular insecticide treatments to control CRW larvae will be allowed on refuge acres. Treating refuges with larval insecticides are needed because of the potential for severe damage and economic impact. However, treating refuges for adult CRW control will be prohibited because adult treatments may diminish the effectiveness of the refuge. If growers spray their corn fields with insecticides to control pests other than CRW, then all acres (Bt and non-Bt) should be treated identically.

Bt fields and the non-Bt refuge acres should be treated with identical agronomic practices such as irrigating all corn (Bt and non-Bt) at the same time. To ensure the production of similar numbers of CRW, Bt and non-Bt corn should be planted in fields with similar backgrounds. For example, if MON 863 hybrids are planted on continuous corn fields then the non-Bt refuge should be planted on continuous corn fields or both should be planted on first-year corn acres. Non-Bt refuges should not be planted on first year corn fields if the MON 863 hybrids are planted on rotated fields.

This interim IRM plan is not intended for fields planted to increase inbred seed since these fields need to be isolated from external corn pollen sources. An in-field or adjacent non-Bt corn refuge would be inconsistent with inbred seed production practices. Monsanto does not believe that planting these fields without an associated refuge will increase the risk of CRW resistance to Cry3Bb because these fields will be planted after soybean and soil insecticides will be applied at planting.

Reviewer's Comments

The 1998 Science Advisory Panel Subpanel concluded that an appropriate resistance management strategy is necessary to mitigate the development of insect resistance to Bt proteins expressed in transgenic crop plants. The 1998 Subpanel recognized that resistance management programs for ECB should be based on the use of both a high dose of Bt and structured refuges designed to provide sufficient numbers of susceptible adult insects. The high dose/refuge strategy assumes that resistance to Bt is recessive and is conferred by a single locus with two alleles resulting in three genotypes: susceptible homozygotes (SS), heterozygotes (RS), and resistant homozygotes (RR). It also assumes that there will be a low initial resistance allele frequency and that there will be extensive random mating between resistant and susceptible adults. Under ideal circumstances, only rare RR individuals will survive a high dose produced by the Bt crop. Both SS and RS individuals will be susceptible to the Bt toxin. A structured refuge is a non-Bt portion of a grower's field or set of fields that provides for the production of susceptible (SS) insects that may randomly mate with rare resistant (RR) insects surviving the Bt crop to produce susceptible RS heterozygotes that will be killed by the Bt crop. This will remove resistant (R) alleles from the insect populations and delay the evolution of resistance. However, it is currently unknown if one or more loci are involved with CRW resistance to Cry proteins or what the initial resistance allele frequency is for these beetles. This information is necessary to determine what dose is needed and what size and structure of refuge is required to delay resistance.

Based on CRW biology, current information provided from simulation models and expected low initial adoption rates (Monsanto suggests <5% in the 1st year, <20% in 2nd year and <40% the third year) and hybrid availability, a 20% non-Bt corn refuge planted with or adjacent to MON 863 corn fields is expected to delay the risk of CRW developing resistance to Cry 3Bb1. Considering the limited movement of CRW larvae, planting refuges close to transgenic fields in large blocks is preferred to narrow strips (Gray 1999, Meinke *et al.* 2001). If a 20% refuge is planted as row strips within a corn field, then at least 6 to 12 consecutive rows should be planted 9 to 18 m from the center of the transgenic corn (Onstad *et al.* 2001). Based on the models discussed above, the "lower" dose of MON 863 seemingly supports a 20% refuge.

WCRW, NCRW and MCRW only feed on a few weedy species other than corn and the value of these alternate hosts as a refuge is not understood (Meinke *et al.* 2001). Therefore, alternate hosts should not be considered as refuge for CRW-protected transgenic corn until more information is available on their effectiveness.

Farmers will expect the option of applying insecticides to treat for CRW adults or larvae on refuge acres because of potential economic loss due to factors such as plant lodging. Insecticides

are applied to the root zone to control larvae and will not affect CRW out of the treated area (Meinke *et al.* 2001). Therefore, it should be assumed that refuge acres will be treated for CRW larvae with chemical insecticides. However, refuge acres should not be treated with insecticides to control CRW adults.

Monitoring:

Summary of Monsanto's Proposal

Changes in susceptibility of target pests to Cry3Bb1 insecticidal protein will be monitored. Baseline susceptibility studies WCRW have been initiated from populations collected in 1999 and 2000 by Dr. Blair Siegfried (University of Nebraska), Dr. Larry Chandler (USDA-ARS) and Dr. Wade French (USDA-ARS). This baseline monitoring was concentrated in areas that are expected to have the greatest risk of resistance, particularly in areas known to have high CRW populations and are expected to have high adoption of MON 863 corn. Populations of WCRW and NCRW were also be collected in 2001 and will be collected in 2002 to determine baseline susceptibility.

Laboratory bioassays using nondiapausing, laboratory-reared WCRW larvae resulted in a estimated LC₅₀ for MON 863 of 75 ppm. Ten populations of field collected WCRW larvae were assayed by Dr. Blair Siegfried (University of Nebraska) and demonstrated an average of twice the susceptibility to MON 863 then the laboratory colonies. Additional WCRW and NCRW larvae collected from the field during 2001 will be assayed in addition to populations that will be collected during the 2002 growing season. Results from the assays will be used to develop baseline susceptibility data.

Reviewer's Comments

CRW management practices can be determined by scouting fields the previous year to estimate damage that will occur the following year (O'Neal *et al.* 2001, Meinke *et al.* 2001). Therefore, sampling fields such as soybean the year before growing corn may provide an indication if CRW control will be needed (O'Neal *et al.* 2001). Insecticide treatments are not typically applied for adult CRW in the eastern Corn Belt; however, broadcast insecticides are used in the western Corn Belt, particularly Nebraska, to control WCRW females and prevent them from laying eggs (Gray 2000). Threshold levels recommended for insecticide applications vary according to region, expert opinion and rotation practices (e.g., first year corn vs continuous corn) (Gray 2000). According to the NCR-46, "[s]couting techniques and thresholds are available for continuous corn, extended diapause of northern corn rootworms, and western corn rootworm oviposition in soybean. These methodologies would enable the use of transgenic varieties in a prescriptive, pest management approach" (Meinke *et al.* 2001). Application of insecticides have been recommended when 0.75 CRW are found per corn plant and 10% of those beetles are gravid females. Monitoring should continue weekly and insecticides should be reapplied if 0.5

beetles per plant are found in the field. Scouting results from mid-July to early September can be used to determine potential larval damage and the need for treatment the following year (Gray and Steffey 1999). Rotating a non-host crop the year following corn is recommended when 0.5 to 1 beetle per plant is found during peak pest population density. The use of root ratings may have also been recommended to predict economic injury levels of CRW. On a scale of 1-6, a mean root rating of >2.5 is expected to lead to economic loss (Levine and Oloumi-Sadeghi 1991). However, studies have shown that root damage ratings should not be used to determine insecticide efficacy or predict yield because results are inconsistent (Spike and Tollefson 1989, Sutter *et al.* 1990, Levine and Oloumi-Sadeghi 1991).

A resistance monitoring strategy for Bt corn is needed to test the effectiveness of resistance management programs. Detecting shifts in the frequency of resistance genes (i.e., susceptibility changes) through resistance monitoring can be an aggressive method to detect the onset of resistance before widespread crop failure occurs. As such, the utilization of sensitive and effective resistance monitoring techniques is critical to the success of an IRM plan. Monitoring techniques such as discriminating dose concentration assays need to be thoroughly investigated for *Diabrotica* spp. for their feasibility as resistance monitoring tools.

Grower participation (e.g., reports of unexpected damage) is an important first step for resistance monitoring. Resistance monitoring is also important because it provides validation of biological parameters used in models. However, resistance detection/monitoring is a difficult and imprecise task. It requires both high sensitivity and accuracy. Good resistance monitoring should have well-established baseline susceptibility data prior to widespread introduction of Bt crops. Although baseline susceptibility data is not completed at this time, research is being conducted to develop the baseline susceptibility of WCRW and NCRW to MON 863. These data are also needed for MCRW.

A comprehensive monitoring plan that targets the CRW and addresses when and where monitoring will occur is needed and should be developed within two years of commercialization. Monitoring will become more important after the accrual of multiple growing seasons of exposure and grower adoption increases. In addition to baseline susceptibility data, information is needed to determine how many individuals need to be sampled and in how many locations. The chances of finding a resistant larvae in a Bt crop depend on the level of pest pressure, the frequency of resistant individuals, the location and number of samples that are collected, and the sensitivity of the detection technique. Therefore, as the frequency of resistant individuals or the number of collected samples increases, the likelihood of locating a resistant individual increases (Roush and Miller 1986). If the phenotypic frequency of resistance is one in 1,000, then more than 3,000 individuals must be sampled to have a 95% probability of one resistant individual (Roush and Miller 1986). Current sampling strategies for ECB have a target of 100 to 200 individuals per location. Previous experience with conventional insecticides has shown that once resistant phenotypes are detected at a frequency $>10\%$, control or crop failures are common (Roush & Miller 1986). Because of sampling limitations and monitoring technique sensitivity, resistance could develop to Bt toxins prior to it being easily detected in the field.

Interim Mitigation/Remedial Action:

Summary of Monsanto's Proposal

Since this IRM plan is limited to three years, Monsanto believes that the risk of resistance is negligible, particularly considering the fact that CRW has one generation per year. If unexpected levels of CRW damage occur during this interim period, growers and/or seed dealers will be instructed to contact Monsanto or an authorized distributor. Upon reports of unexpected CRW damage in MON 863 corn hybrids, Monsanto will sample local pest populations and conduct necessary *in vitro* and *in planta* assays. If presence of the Cry3Bb1 protein is confirmed, then studies will be conducted to determine if the level of susceptibility of the field collected insects differs from the baseline level. Discriminating concentration bioassays will be used when possible to determine if levels of susceptibility have increased.

If discriminating concentration bioassays are unavailable, insect resistance will be determined from neonate bioassays of progeny of the sampled pest population. In the absence of the discriminating concentration bioassay, Monsanto will determine if CRW susceptibility to MON 863 is increased if neonate larvae exhibit both of the following two criteria. 1. "An LC_{50} in a standard diet bioassay (incorporating the Cry3Bb protein) that exceeds the upper limit of the 95% confidence interval of the mean historical LC_{50} for susceptible pest populations, as established by the baseline measurements." 2. "Over 50% of Cry3Bb-expressing plants with one or more root nodes destroyed under controlled laboratory conditions." The definition of confirmed resistance may evolve and change as new information becomes available.

Any instance of confirmed CRW resistance will be reported to the Agency within 30 days and mitigation measures will be reported within 90 days. Mitigation measures will include: 1. Informing growers and extension specialists in the affected area of the confirmed report of CRW resistance. 2. Sales in the affected area will be terminated immediately. 3. Alternate CRW control measures will be recommended to growers and extension specialists in the affected areas. Monsanto will also notify the Agency of a long term IRM strategy for an affected area within 90 days of confirmed resistance and work closely with the Agency to mitigate resistance.

As part of a mitigation action plan, Monsanto will initiate some or all of the following actions. Growers and extension agents in the affected areas will be informed of the resistance incidence and monitoring will be increased in that area. Alternative CRW control measures will be recommended and intensified IRM measures will be instituted. If these measures do not mitigate resistance, Monsanto will cease sales in the affected area until the Agency agrees that an effective IRM plan has been implemented.

Reviewer's Comments

There is a concern regarding Monsanto's outline of detecting and confirming resistance. Monsanto suggests that they will initiate mitigation measures when unexpected levels of CRW damage occur. However, Monsanto does not describe what is meant by unexpected levels of damage. Some level of damage is expected since there is not a high dose of MON 863 expressed to control the CRW and research has shown that some level of "grazing" will occur. Monsanto must identify what unexpected levels of damage are and relay this information to EPA, seed dealers, extension specialists, NCGA and growers. This may be accomplished in many ways

including websites and Technology Use Guides.

Confirmed reports of pest resistance should be reported to the Agency as soon as possible but must be within 30 days. Once resistance has been confirmed, alternative control measures to reduce or control the local target pest population should be recommended to customers, extension agents, consultants, university cooperators, seed distributors, processors, state regulatory authorities, EPA regional and national authorities, and any other pertinent personnel of the incidence(s) of resistance in the affected area. Where appropriate, customers and extension agents in the affected area should apply insecticides and/or crop rotation practices to control any potentially resistant individuals.

As soon as possible following confirmation of resistance, but must be within 90 days of a confirmed instance of pest resistance, as defined above, Monsanto should notify the Agency of the immediate mitigation measures that were implemented and submit a proposed long-term resistance management action plan for the affected area. Monsanto should work closely with the Agency in assuring that an appropriate long-term resistance management action plan for the affected area is implemented. An action plan that is approved by EPA should be implemented that consists of some or all the following elements, as warranted: 1) Inform customers and extension agents in the affected area of pest resistance; 2) Increase monitoring in the affected area, and ensuring that local target pest populations are sampled on an annual basis; 3) Recommend alternative measures to reduce or control target pest populations in the affected area; 4) Implement intensified local IRM measures in the affected area based on the latest research results. The implementation of such measures will be coordinated by the Agency with other registrants; and 5) Monsanto should cease sales of all MON 863 Bt corn hybrids until resistance has been shown to have been abated. During the sales suspension period, Monsanto may sell and distribute in these counties only after obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy should be coordinated with the Agency.

For the growing season(s) following a confirmed resistance incident(s), Monsanto should maintain the sales and distribution suspension of all MON 863 hybrids potentially affected by the resistant pest populations or areas in which resistance is considered to be serious. This must be done within the affected region or if undetermined, the affected county(ies) and proximate surrounding counties. This sales suspension should remain in place until resistance has been determined to have subsided (within 5 to 10% or one standard deviation of baseline levels). In addition, Monsanto should develop, recommend, and implement alternative resistance management strategies for controlling the resistant pest(s) on corn with all necessary personnel (e.g. growers, extension agents, consultants, seed distributors, processors, university cooperators, and state/federal officials) in the affected region/county(ies) and surrounding counties of the resistance situation. All necessary personnel (e.g. growers, consultants, extension agents, seed distributors, processors, university cooperators, and state/federal authorities) in the affected region/county(ies) and surrounding counties of the resistance situation should be informed. Monitoring and surveillance in the affected area(s) for resistance and define the boundaries of the affected region should be intensified and studies on the rate of decline of resistance in the field should be conducted. Monsanto should continue to work with the Agency, states, grower groups, extension agents, consultants, university cooperators, or other expert personnel and other

stakeholders to insure the implementation and development of appropriate mitigation measures for resistance in the affected areas.

If EPA agrees that an effective local resistance management plan has been implemented which mitigates resistance, the registrants can resume sales in the affected county(ies).

Grower Adoption and Hybrid Availability:

Summary of Monsanto's Proposal

Monsanto anticipates that initial adoption rates of MON 863 corn will be low relative to the potential market share at full maturity. With any new technology, initial grower adoption rates are initially low and increase over time (Grieshop *et al.* 1988). Effectiveness of the technology, information dissemination, hybrid availability and grower characteristics influence the growers level of awareness, interest, evaluation, trial and eventual adoption of MON 863 corn. Growers will also probably utilize other CRW control techniques that will limit adoption of MON 863 over time particularly as seed treatments and other transgenic products become available. Adoption rates will be further limited by the lack of approval for MON 863 corn in the European Union, thus restricting sales to domestic and other approved outlets. The European Union will probably not approve the sale of MON 863 corn during the interim three years requested by Monsanto.

Growers do not typically plant a single corn hybrid. Doane (2000) showed that an average of 3.4 hybrids purchased from 1.6 suppliers are planted by growers. In addition, research and seed company experience indicate that growers do not commit to large volumes of a particular hybrid until the test it first. Currently registered transgenic crops including Roundup ready soybeans, Roundup Ready corn and Yieldgard corn were initially adopted at low rates and incrementally increased over time. In general, growers will chose their seed hybrids based on confidence in performance and yield.

MON 863 will be sold in Monsanto's Asgrow and DEKALB corn hybrids in addition to being licensed to other seed companies. Monsanto expects MON 863 to be available from less than 50% of the total market share of seed companies. Seed availability and number of corn hybrids containing MON 863 will be limited initially due to breeding and manufacturing limitations and will gradually increase during the first three years of registration. According to Monsanto, four to five years are needed for a company to be able to offer all hybrids as transgenic corn.

Reviewer's Comments

Although Monsanto anticipates grower adoption rates to be slow initially (<5% the 1st year, <20% the 2nd year and <40% the 3rd year), the Agency acknowledges that there is no guarantee of how quickly growers will adopt CRW-protected transgenic corn. According to Dr. Michael Gray (University of Illinois), adoption of CRW protected transgenic corn is expected to occur much more quickly than the lepidopteran-active transgenic corn was adopted by growers. Gray suggests that growers are likely to accept the costs of growing transgenic corn cultivars to control CRW in order to reduce the costs associated with soil insecticide applications (Gray 1999).

Grower Education:

Summary of Monsanto's Proposal

Growers will be required to sign a legal contract in which they agree to adhere to IRM requirements. An education program will be initiated by Monsanto to inform growers of IRM requirements, why they are important and what individual grower responsibilities are. Information will be available to growers in various ways, but primarily from the Technology Use Guide. The Technology/Stewardship Agreement signed by growers will inform them of IRM requirements outlined in the Technology Use Guide. Signed Technology Agreements will inform growers that access to MON 863 will be lost if IRM requirements are not adhered to. Additional information to the Technology Use Guide will be mailed to growers as new information is available.

Monsanto also intends to develop a comprehensive stewardship training course that will provide growers planting MON 863 corn with information on IRM requirements and grower responsibilities. In addition, seed sales representatives will be trained to address IRM requirements as part of their selling endeavors. This approach to grower education should be useful since many growers have indicated that they receive most of their IRM information from seed dealers. Monsanto will also work with the NCR-46, American Crop Protection Association (ACPA), Biotechnology Industry Organization (BIO), the National Alliance of Independent Crop Consultants, extension specialists, United States Department of Agriculture (USDA), EPA, National Corn Growers Association (NCGA) as well as state and county corn growers associations and other relevant groups.

A survey will be sponsored by Monsanto and conducted by an independent firm to determine if growers are receiving the necessary IRM information and implementing IRM. This survey should aid in improving education and compliance efforts. A coalition of stakeholders (e.g., NCGA and extension specialists) will be formed by Monsanto to address CRW IRM issues including efforts in grower education.

Reviewer's Comments

Growers are perhaps the most essential element for the implementation and success of any IRM plan as they will ultimately be responsible for ensuring that refuges are planted according to guidelines and that *Bt* fields are monitored for unexpected pest damage. Therefore, a program that educates growers as to the necessity of IRM and provides guidance as to how to deploy IRM should be an integral part of any resistance management strategy. The 2000 SAP also suggested that a comprehensive education program may help increase IRM compliance (SAP 2001). Ideally, the educational messages presented to growers should be consistent (among different registrants if applicable for CRW) and reflect the most current resistance management guidelines. Specific examples of education tools for growers can include grower guides, technical bulletins, sales materials, training sessions, Internet sites, toll-free numbers for questions or further information, and educational publications.

Grower Compliance:

Summary of Monsanto's Proposal

Adhering to IRM requirements economically impacts growers based on monetary and time costs. According to Monsanto, if IRM requirements are too complex or time consuming to initiate, growers may avoid planting Bt corn. The rate CRW resistance to MON 863 develops may be impacted by growers farming and Agronomic practices. The 1998 Scientific Advisory Panel (SAP) on IRM for Bt crops acknowledged the importance of creating an IRM plan that is feasible for growers to institute. In addition, ILSI-HESI concluded that "the IRM plan should be cost-effective, flexible, easily adopted, and compatible with common production practices." Based on conclusions of these expert Panels as well as grower surveys, Monsanto acknowledges a need for multiple IRM strategy options available for different types of fields and farming practices.

According to Monsanto, refuge size should be based on the goals of IRM as well as grower economic considerations. Due to the potential economic losses, it is important for growers to be allowed to spray their refuges with insecticides when economic thresholds of CRW larvae occur. Although growers may spray their refuges, insecticide use will still be significantly reduced in areas CRW protected corn is grown. Grower surveys conducted by independent groups and sponsored by Monsanto between the years of 1997 and 2000 indicate that growers need a variety of options to implement IRM requirements regardless of farm size. There is also information that suggests that a grower may implement more than one design when planting a refuge. If refuge designs are too restrictive, surveys indicate that growers will be less likely to plant CRW protected Bt corn. In general, refuge requirements need to be kept simple, practical and flexible. A survey conducted by Monsanto in 2000 concluded that growers considered a 20% non-Bt refuge as generally acceptable, but many expressed a concern that a 30% refuge may not be feasible. Growers also preferred to plant their refuge as a seed mix; however, a seed mix is not being recommended by Monsanto at this time.

There are several reasons identified by Monsanto that explain why restricting refuges for MON 863 corn to in-field strips and limiting adjacent blocks as an option may be too difficult or unmanageable for many growers. Most fields are managed as whole production units that are rotated annually. Production practices are dependent on unpredictable impacts such as weather so flexibility and the ability to modify planting schemes are necessary. As farming efficiency has improved, the use of bulk seed handling and planting systems are being increasingly adopted, thus making it difficult for growers to micromanage a field. A quick and simple planting operation is needed by growers since there is a short time-period in the Spring when fields are planted.

Reviewer's Comments

Evaluation of grower compliance is largely based on results of grower surveys. Surveys indicate that 100% compliance is not likely and that some level of non-compliance must be expected. An expectation of 30% (or greater) non-compliance may be reasonable, given survey results for lepidopteran protected corn. However, the 2000 SAP indicated that while surveys such as these

are useful for tracking grower attitudes, they are not reliable for determining actual grower compliance (SAP 2001). The format of the surveys (mail or phone interviews) may encourage non-compliant growers to misrepresent their actions or “cheat” in their responses. Without confirmatory visits to individual farms (i.e., audits), it may be impossible to verify the accuracy of grower responses. The end result could be increased “false-positives,” which may artificially inflate estimates of grower compliance. As such, actual non-compliance may be significantly higher than the survey results would suggest. To resolve this problem, the 2000 SAP suggested utilizing surveys created and conducted by independent parties to assess grower practices (SAP 2001). In addition to this recommendation, it may be useful to conduct some on-farm visits for firsthand verification of compliance. Such visits could be performed as part of a survey process, to evaluate the accuracy of grower survey responses.

Grower compliance is a greater concern because Monsanto is proposing an interim IRM plan which may be amended as new information becomes available. If the IRM requirements change based on additional information gotten during the interim period, it may be problematic to get the new information to growers and grower compliance with any new requirements may decline. Therefore, growers should sign agreements every year they grow MON 863 and growers should be required to read the Technology Use Guide prior to signing agreements.

The following requirements should be adopted to help ensure a high level of grower compliance with IRM requirements. To avoid confusing or discouraging growers, new IRM programs should be kept simple and consistent with existing programs so that growers will not be discouraged from properly implement IRM or will not grow transgenic crops. Growers should be required to sign a technology use agreement that outlines IRM requirements and acknowledges the growers responsibility to comply with them on an annual basis. The agreement will also state that growers received the Product Use Guide. This agreement may be a section of the growers order sheet or some other document or format. An annual industry-supported survey conducted by a third party should be submitted to the Agency as a tool to monitor grower compliance. Additional education efforts should target non-compliant growers and access to the technology will be limited for growers found to be non-compliant.

Monsanto's Conclusions:

The use of MON 863 corn to control CRW larvae will be easier to use and safer than chemical insecticide control. Both growers and consumers will benefit from the reduction in insecticide applications. An IRM plan should not limit the use of MON 863 corn; rather, an IRM plan should be flexible, not overly encumber growers and fit into differing field situations and agronomic practices. It is also important for the plan to allow for the utilization of IPM practices. Monsanto suggests that refuge acres should be planted where CRW-infestations are low and insecticide use will be low. Planting MON 863 corn in areas with lower CRW larval infestations should lead to an increase in adult productivity and survival. Aspects of pest biology, grower needs, market penetration and impacts on growers were all considered in Monsanto's interim IRM plan.

Monsanto will continue to sponsor research intended to be used to develop a long-term IRM plan (Table 1). In populations and dispersal. Feasibility and grower compliance of IRM strategies

for MON 863 corn will also be evaluated during the interim registration period. An interim period is necessary to allow planting of MON 863 corn, conduct research and evaluate IRM strategies.

Reviewer's Conclusions:

Currently, there is not enough information available to definitively determine the size and structure of a non-Bt corn refuge for MON 863 needed to appropriately delay the development of CRW resistance. In a memorandum dated April 12, 2002 and e-mailed to the Agency by Russ Schneider (Monsanto Co.), the Canadian Food Inspection Agency (CFIA) states that the current Monsanto IRM plan is based on a high dose product for ECB control; however, MON 863 does not provide a high dose. Therefore, the CFIA concluded that "[i]t is currently uncertain whether requirements for refuge placement and the proportion of field planted to a refuge will be similar or different to that in place for ECB hybrids. The Agency acknowledges that there is a lack of information on dose and pest biology; therefore, a conservative approach to CRW IRM is necessary. Current information suggests that MON 863 provides a low to moderate dose of Cry3Bb1 for CRW.

The Agency agrees with the following recommendations quoted from the CFIA April 12, 2002 memo. "1. The refuge of a non-Bt corn hybrid consists of no less than 20% of the total corn planted in a field. 2. The refuge and Bt planting areas should both have the same crop rotation histories. 3. The refuge is located within a field with a flexible configuration of non-Bt rows planted to convenience the grower and orientation of the field. 4. Where circumstances require that a refuge cannot be planted within a field, the refuge must be planted adjacent to the Bt field. 5. The refuge may be treated with a banded soil insecticide for larval rootworm control."

Based on current information on CRW biology, MON 863 dose, simulation models, hybrid availability and adoption rate, a 20% refuge should be adequate to produce enough CRW adults to delay resistance. In addition, planting refuges adjacent to or within MON 863 corn fields should allow random mating of CRW even though there is limited movement of larvae and virgin females.

Monsanto expects the number of acres of MON 863 corn will be limited in each state during the first three years of registration because of a lack of hybrid availability and slow grower adoption rates as they evaluate the new technology. Therefore, Monsanto suggests that there will potentially be a greater than 20% refuge leading to a decrease in the risk of resistance. Although Monsanto does not plan to plant a refuge for inbred seed increase fields, they will treat with insecticides that will probably control any resistant CRW adults that may emerge. It is, therefore, acceptable to plant inbred seed increase acres without a refuge.

Planting a limited number of acres per state of MON 863 corn with a 20% non-Bt corn refuge planted adjacent to the Bt field or as strips within the field should be adequate to ensure resistance does not develop during the three year interim period requested by Monsanto. Although unmated adult CRW females have been shown to move up to ten rows, it may not be practical for growers to plant ten row strips. Therefore, at least six rows (but preferably 12 rows) of non-Bt corn should be planted when in-field strips are utilized as refuge (Onstad *et al.* 2001).

In addition, refuges should be planted with similar hybrids as the MON 863 field and identical agronomic practices should be employed for the transgenic and non-transgenic fields to ensure simultaneous adult emergence.

The NCR-46 committee which consists of research and extension CRW specialists as well as other cooperators commented on Monsanto's interim IRM plan at the request of the BPPD IRM Team. This group is recognized at the national authorities on CRW biology, ecology and management. The NCR-46 agrees that the potential for CRW to develop resistance to MON 863 during a three year interim period with low adoption rates is negligible. Since there is not a high dose of MON 863, there will be a significant number of CRW larvae survival. In addition, evidence of changes in larval feeding behavior on MON 863 corn will lead to increased larval survivorship. This increase in larval survivorship will result in a decreased chance of resistance.

Monsanto claims that MON 863 controls CRW (*Diabrotica* spp.). This implies that all CRW species including WCRW, NCRW, MCRW and SCRW will be controlled by MON 863. However, in their "interim IRM plan", Monsanto does not address the Mexican corn rootworm or southern corn rootworm. Information on pest biology and refuge structure is needed for a general claim of *Diabrotica* spp. control. Additional information is needed on various aspects of CRW pest biology as it relates to an IRM strategy.

Monsanto also does not adequately address grower education or mitigation measures if resistance occurs. A detailed grower education program is necessary to ensure grower compliance, particularly since the IRM requirements may change as more information becomes available. Growers should be required to sign a Technology/Stewardship Agreement each year they intend to plant MON 863 corn to ensure they are aware of the current IRM requirements.

There is also concern regarding Monsanto's outline of detecting and confirming resistance. Monsanto suggests that they will initiate mitigation measures when unexpected levels of CRW damage occur. However, Monsanto does not describe what is meant by unexpected levels of damage. Some level of damage is expected since there is not a high dose of MON 863 expressed to control the CRW and research has shown that some level of "grazing" will occur. Monsanto must identify what unexpected levels of damage are and relay this information to EPA, seed dealers, extension specialists, NCGA and growers. This may be accomplished in many ways including websites, a 1-800 telephone number and Technology Use Guides. Further investigation into confirming resistance is also needed. Monsanto's time-line includes identifying the insects as resistant, confirming the plant is expressing MON 863 and taking mitigation measures within 90 days. After this length of time, the growing season will likely be over and it will be too late for mitigation measures. It is, therefore, necessary for Monsanto to cease sales in an affected area after a confirmed report of resistance. The area and amount of time (in years) that sales should cease should be determined in consultation with EPA.

In addition, pages 6 and 9 of the IRM submission states that "[h]ybrids containing event MON 863 will be released both as stand-alone products and in combination with the *cry1Ab* gene for lepidopteran control because of the combined insect management needs of many farmers." However, the risk of resistance from a Cry1Ab/Cry3Bb stacked product has not been determined. Prior to the release and distribution of a Cry1Ab/Cry3Bb1 "stacked" product, an

acceptable IRM plan specifically designed for a “stacked” product must be submitted to the Agency for review. Although refuges are similar for the lepidopteran protected and coleopteran protected Bt corn, Monsanto should consult with the Agency prior to distributing combination products.

References:

Andow, D. A. and D. N. Alstad. 2002. Preliminary draft: efficacy of between field refuges for resistance management of transgenic corn rootworm insecticidal corn: model results. MRID 455770-01

Ball, H. J. and G. T. Weekman. 1963. Differential resistance of corn rootworms to insecticides in Nebraska and adjoining states. J. Econ. Entomol. 56: 553-555.

Ball, H. J. 1983. Aldrin and dieldrin residues in Nebraska (USA) soils as related to decreasing LD₅₀ values for the adult western corn rootworm (*Diabrotica virgifera*), 1962-1981. J. Environ. Sci. Heal. Part B Pestic. Food Contam. And Agri. Wastes. 18: 735-744.

Bergman, M. K. and F. T. Turpin. 1984. Impact of corn planting date on the population dynamics of corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 13: 898-901.

Boetel, M. A. and B. W. Fuller. 1997. Seasonal emergence-time effects on adult longevity, fecundity, and egg viability of northern and western corn rootworms (Coleoptera: Chrysomelidae). Environ. Entomol. 26: 1208-1212.

Branson *et al.* 1980

Branson, T. F. and J. L. Krysan. 1981. Feeding and oviposition behavior and life cycle of *Diabrotica*: an evolutionary view with implications for pest management. Environ. Entomol. 10(6): 826-831.

Branson, T. F., J. R. Reyes, and H. M. Valdes. 1982. Field biology of Mexican corn rootworm, *Diabrotica virgifera zea* (Coleoptera: Chrysomelidae), in central Mexico. Environ. Entomol. 11: 1078-1083.

Branson, T. F. and G. R. Sutter. 1985. Influence of population density of immatures on size, longevity, and fecundity of adult *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Environ. Entomol. 14: 687-690.

Coates, S. A., J. J. Tollefson and J. A. Mutchmor. 1986. Study of migratory flight in the western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 15: 1-6.

Davis, P. M., N. Brenes and L. L. Allee. 1996. Temperature dependent models to predict regional difference in corn rootworm (Coleoptera: Chrysomelidae) phenology. Environ. Entomol. 25: 767-775.

Doane 2000

Dudin, Y. A., B-P. Tonnu, L. D. Albee and R. P. Lirette. 2001. Amended report for MSL 16559: *B.t.* Cry3Bb1.11098 and NPTII protein levels in tissue samples collected from corn event MON 863 grown in 1999 field trials. Report No. MSL-17181; an unpublished study conducted by Monsanto Company. Submitted to EPA on May 21, 2001, MRID 454240-01.

Elliott, N. C., G. R. Sutter, T. F. Branson and J. R. Fisher. 1989. Effect of population density of immatures on survival and development of the western corn rootworm (Coleoptera: Chrysomelidae). *J. Entomol. Sci.* 24: 209-213.

Elliot, N. C. and G. L. Hein. 1991. Population dynamics of the western corn rootworm: formulation, validation, and analysis of a simulation model. *Ecol. Modeling.* 59: 93-122.

FIFRA Scientific Advisory Panel (SAP). 1998. Subpanel on *Bacillus thuringiensis* (*B.t.*) Plant-Pesticides and Resistance Management. A report prepared for the Environmental Protection Agency, dated February 9-10, 1998. (Docket No.: OPP 00231).

Gray, M. E. 1999. Transgenic insecticidal cultivars for corn rootworms: resistance management considerations. *In* Proceedings of the Crop Protection Technology Conference. University of Illinois, Urbana-Champaign. pp 50-55.

Gray, M. E. 2000. Prescriptive use of transgenic hybrids for corn rootworms: an ominous cloud on the horizon? *In* Proceedings of the Crop Protection Technology Conference. University of Illinois, Urbana-Champaign. pp. 97-103.

Gray, M. E., E. Levine and H. Oloumi-Sadeghi. 1998. Adaptation to crop rotation: Western and northern corn rootworms respond uniquely to a cultural practice. *Recent Res. Devel. Entomol.* 2: 19-31.

Gray, M. E. and K. L. Steffey. 1999. Insect pest management for field and forage crops. *In* Illinois Agricultural Pest Management Handbook, University of Illinois, Urbana-Champaign.

Gustin, R. D. and T. E. Schumacher. 1989. Relationship of some soil pore parameters to movement of first-instar western corn rootworm (Coleoptera: Chrysomelidae). *Environ. Entomol.* 18: 343-346.

Hill, R. E. and Z. B. Mayo. 1980. Distribution and abundance of corn rootworm species as influenced by topography and crop rotation in eastern Nebraska, USA. *Environ. Entomol.* 9: 122-127.

International Life Science Institute Health and Environmental Sciences Institute (ILSI-HESI) 1999. An evaluation of insect resistance management in Bt field corn: a science-based framework for risk assessment and risk management. Report of an expert panel. November 23, 1998.

- Isard, S. A., M. A. Nasser, J. L. Spencer and E. Levine. 1999. The influence of weather on western corn rootworm flight activity at the borders of a soybean field in east central Illinois. *Aerobiologia*. 15: 95-104.
- Isard, S. A., J. L. Spencer, M. A. Nasser and E. Levine. 2000. Aerial movement of western corn rootworm (Coleoptera: Chrysomelidae) : diel periodicity of flight activity in soybean fields. *Environ. Entomol.* 29(2): 226-234.
- Jackson, J. J. and N. E. Elliott. 1988. Temperature-dependent development of immature stages of the western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae): description, distribution, and sexual compatibility. *Ann. Entomol. Soc. Amer.* 73: 123-130.
- Krysan, J. L., J. J. Jackson and A. C. Lew. 1984. Field termination of egg diapause in *Diabrotica* with new evidence of extended diapause in *D. barberi* (Coleoptera: Chrysomelidae). *Environ. Entomol.* 13: 1237-1240.
- Krysan, J. L., D. E. Foster, T. F. Branson, K. R. Ostlie, and W.S. Cranshaw. 1986. Two years before the hatch: rootworms adapt to crop rotation. *Bull. Entomol. Soc. Amer.* 32: 250-253.
- Knutson, J. L., B. E. Hibbard, B. D. Barry, V. A. Smith and L. L. Darrah. 1999. Comparison of screening techniques for western corn rootworm (Coleoptera: Chrysomelidae) host plant resistance. *J. Econ. Entomol.* 92: 714-722.
- Levine, E. and H. Oloumi-Sadeghi. 1991. Management of Diabroticite rootworms in corn. *Annu. Rev. Entomol.* 36: 229-255.
- Levine, E., H. Oloumi-Sadeghi and J. R. Fisher. 1992a. Discovery of multiyear diapause in Illinois and South Dakota northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. *J. Econ. Entomol.* 85(1): 262-267.
- Levine, E., H. Oloumi-Sadeghi and C. R. Ellis. 1992b. Thermal requirements, hatching patterns, and prolonged diapause in western corn rootworm (Coleoptera: Chrysomelidae) eggs. *J. Econ. Entomol.* 85(6): 2425-2432.
- Levine, E. and H. Oloumi-Sadeghi. 1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn for seed production following soybean grown for seed production. *J. Econ. Entomol.* 89: 1010-1016.
- Meinke, L. J., B. D. Siegfried, R. J. Wright and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm populations to selected insecticides. *J. Econ. Entomol.* 91: 594-600.
- Metcalf, R.L. 1983. Implications and prognosis of resistance to insecticides. *In* Pest Resistance to Pesticides. G. P. Georgiou and T. Sato [eds.]. pp. 703-733. Plenum Press, New York.
- Metcalf, R. L. 1986. Foreword. *In* Methods for the study of pests *Diabrotica*, pp. Vii-xv. J. L.

Krysan and T. A. Miller (eds.). New York, Springer-Verlag. 260 pp.

Miota, F. M. E. Scharf, M. Ono, P. Marcon, L. J. Meinke, R. J. Wright, L. D. Chandler and B. D. Siegfried. 1998. Mechanism of methyl and ethyl parathion resistance in western corn rootworm (Coleoptera: Chrysomelidae). *Pest. Biochem. Physiol.* 61: 39-52.

O'Neal, M. E., M. E. Gray and C. A. Smyth. 1999. Population characteristics of a western corn rootworm (Coleoptera: Chrysomelidae) strain in east-central Illinois corn and soybean fields. *J. Econ. Entomol.* 92(6): 1301-1310.

O'Neal, M. E., M. E. Gray, S. Ratcliffe and K. L. Steffey. 2001. Predicting western corn rootworm (Coleoptera: Chrysomelidae) larval injury to rotated corn with Pherocon AM Traps in soybean. *J. Econ. Entomol.* 94(1): 98-105.

Onstad, D. W., C. A. Guse, J. L. Spencer, E. Levine and M. E. Gray. 2001. Modeling the dynamics of adaptation to transgenic corn by western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 94(2): 529-540.

Ostlie, K. 2001. Crafting crop resistance to corn rootworms. *Nat. Biotech.* 19: 624-625.

Naranjo, S. E. and A. Sawyer. 1988. Impact of host plant phenology on the population dynamics and oviposition of northern corn rootworm. *Diabrotica barberi* (Coleoptera: Chrysomelidae), in field corn. *Environ. Entomol.* 17: 508-521.

Roush, R. T., and G. L. Miller, 1986. Considerations for design of insecticide resistance monitoring programs. *J. Econ. Entom.* 79: 293-298.

Short, D. E. and R. J. Luedtke. 1970. Larval migration of the western corn rootworm. *J. Econ. Entomol.* 63: 325-326.

Spike, B. P. and J. J. Tollefson. 1989. Relationship of root ratings, root size, and root regrowth to yield of corn injured by western corn rootworm (Coleoptera: Chrysomelidae). 82(6): 1760-1763.

Stavisky, J. and P. M. Davis. 1998. The effects of corn maturity class on western corn rootworm (Coleoptera: Chrysomelidae) phenology. *J. Kans. Entomol. Soc.* 70: 261-271.

Strnad, S. P., M. K. Bergman and W. C. Fulton. 1986. First-instar western corn rootworm (Coleoptera: Chrysomelidae) response to carbon dioxide. *Environ. Entomol.* 15: 839-842.

Strnad, S. P. and M. K. Bergman. 1987. Movement of first-instar western corn rootworms (Coleoptera: Chrysomelidae) larvae in corn roots. *Environ. Entomol.* 16: 1193-1198.

Strnad, S. P. and M. K. Bergman. 1987. Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. *Environ. Entomol.* 16: 1193-1198.

Sutter, G. R., J. R. Fisher, N. C. Elliott and T. F. Branson. 1990. Effect of insecticide treatments on root lodging and yields maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). *J. Econ Entomol.* 83(6): 2414-2420.

Suttle, P. J., G. J. Musick and M. L. Fairchild. 1967. Study of larval migration of the western corn rootworm. *J. Econ. Entomol.* 60: 1226-1228.

Tollefson, J. J. 1988. A pest adapts to the cultural control of crop rotation. *Proc. Brit. Crop Prot. Conf.* 3: 1029-1033.

Van Woerkom, G. J., F. T. Turpin and J. R. Barrett, Jr. 1983. Wind effect on western corn rootworm (Coleoptera: Chrysomelidae) flight behavior. *Environ. Entomol.* 12: 196-200.

Woodson, W. D. and L. D. Chandler. 2000. Effects on development of immature Mexican corn rootworm (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Amer.* 93(1): 55-58.