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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

NOV 22 2011

Decision: 441144, 447967
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SUBJECT: Updated BPPD IRM Review of Reports of Unexpected Cry3Bb1 Damage, Monsanto's 2009 Corn Rootworm Monitoring Report, and Revised Corn Rootworm Resistance Monitoring Plan for MON 88017, MON 88017 x MON 810, MON 863, MON 863 x MON810, MON 89034 x TC1507 x MON 88017 x DAS-59122-7, and MON 89034 x MON 88017 (EPA Reg. Nos. 524-551, 524-552, 524-528, 524-545, and 68467-7); MRIDs 478846-01 and 478875-03.

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Actions Requested:

The BPPD IRM Team has been asked to review and respond to Monsanto's resistance monitoring data from 2009 for *Diabrotica virgifera virgifera* LeConte (Western corn rootworm, WCRW), which was submitted for *Bt* protected corn registrations expressing Cry3Bb1 as a condition of registration (MRID 482080-01) as well as Monsanto's response to EPA's request for additional information (MRID 484368-01). Furthermore, this memorandum addresses multiple reports of unexpected damage in Cry3Bb1 corn and provides the conclusions and recommendations of the BPPD IRM Team.

This review has been updated from the first draft (dated September 29, 2011) to make a number of technical corrections.

EXECUTIVE SUMMARY

The BPPD IRM Team concludes that Cry3Bb1 resistance is suspected in at least some portions of four states in which "unexpected damage" reports originated (IA, IL, MN, and NE) and recommends that the Cry3Bb1 remedial action plan be implemented for "suspected resistance". The BPPD IRM Team's conclusions are based on multiple documented cases of unexpected "severe" corn rootworm damage to Cry3Bb1 fields (Gassmann et al. 2011a; Gray 2011a, c) and other undocumented reports from corn entomologists.

The 2009 resistance monitoring data for Cry3Bb1 showed that field-collected populations (collected from Illinois, Iowa, and Nebraska) were less susceptible to the toxin than the laboratory reference colony. In 2009, every field-collected population had a higher EC_{50} and LC_{50} than the laboratory control, in some cases by an order of magnitude. Sampling was conducted in three states (Nebraska, Illinois, and Iowa); the BPPD IRM Team recommends that Colorado, South Dakota, Minnesota, and western Wisconsin be included in future monitoring based on the registrant's reported Cry3Bb1 performance inquiries.

After reviewing Monsanto's overall resistance monitoring strategy for Cry3Bb1 (including responses to previous Agency reviews), the BPPD IRM Team concludes that the registrant's current resistance monitoring program (as proposed) is inadequate and likely to miss early resistance events. This is due to a sampling trigger (> 1.5 NIS) in fields with unexpected damage that is too high (and may miss potentially resistant populations) and a sampling regime that collects beetles too far (1-2 miles away) from problem fields.

CONCLUSIONS AND RECOMMENDATIONS

1. THE BPPD IRM TEAM REVIEW OF MONSANTO'S MONITORING PLAN AND RESPONSE TO BPPD (2010)

1a) After reviewing Monsanto's monitoring plan, the BPPD IRM Team concludes that the registrant's current resistance monitoring program (as proposed) is inadequate and likely to miss early resistance events. This conclusion was based on the following observations:

- The registrant proposed to collect insects from problem fields within 1-2 miles from neighboring sites of failed fields. The BPPD IRM Team believes that this proposal is insufficient if the goal is to determine whether *Bt* failure is due to resistance of CRW population. Since the majority of adult corn rootworm may not disperse long distances, the greatest probability of capture of resistant genotypes should be in the problem fields, possibly in adjacent fields, but less likely in fields 2 miles away during that particular year. To collect in neighboring sites is reasonable after infield collections have occurred or during the following year because of possible adult movement. It is imperative that the language for this crucial procedure is unambiguous and clear and that Monsanto and EPA come to a timely agreement on the method of collecting adult CRW in problem fields. If the resistance monitoring program is to be meaningful, a more proactive, effective approach must be adopted.

- The BPPD IRM Team concludes that Monsanto's Greater Than Expected Damage (GTED) threshold of 1.5 (Node Injury Scale, NIS) for a single toxin PIP with non-high dose expression is generally too high. The BPPD IRM Team stated in the 2010 review that injury ratings of 1.0 should trigger investigations. Ideally, a comparison should occur between performance of *Bt* and nearby refuge corn; however, this may not be an option if growers were non-compliant with refuge requirements. In such non-compliant

cases, the probability that severe root damage is due to resistance likely is greater and, therefore, the BPPD IRM Team considers a threshold level of 1.0 for a single toxin PIP a more defensible (conservative) trigger to initiate adult collections. During years with great pest pressure (not associated with agronomic practices such as growing continuous corn for years), it may be reasonable to deviate from the 1.0 threshold and use 1.5 (based on a graded scale where *Bt* damage is related to adult density and damage to refuge corn). The BPPD IRM Team concludes that for SmartStax products (i.e. SSX RIB), a threshold of 1.0 (as proposed by Monsanto) is not conservative enough; this was already discussed in BPPD (2010). The pyramid has greater activity against CRW and should, therefore, have a lower threshold (such as 0.5) to trigger beetle collections. Additionally, in a seed blend it will be inherently more difficult to detect *Bt* performance issues, therefore, it may not be realistic to use the following threshold "50% of the plants must show greater than 0.5 (NIS) damage" before an investigation occurs. The BPPD IRM Team proposes that any clearly visible sign of damage exceeding the threshold level (0.5) in a seed blend (after trait expression has been confirmed and other causes ruled out, but irrespective of the percentage of *Bt* plant damage in that field) trigger adult collections.

- If Monsanto was unable to collect adults from problem fields because they were contacted too late by seed dealers, then it is important that adult sampling occurs in the problem areas the following season irrespective of pest pressure and damage in the old problem fields. If growers sprayed their fields after they observed high densities of adults during a problem year or used other methods of control, then the pest pressure and subsequent *Bt* damage should be less severe the following year. If Monsanto does not collect in such problem areas during the following season because of "below threshold damage" in *Bt* fields (1.0 for single PIPs and 0.5 for SSX), then they will miss early resistance events in those areas. If there is resistance but low pest pressure, damage can be expected to be lower than in cases where there is high pest pressure coupled with resistant individuals in the population.

1b) Monsanto reported that high rootworm pressure was typically the result of continuous-corn plantings that built up CRW population densities over time and used this reasoning as a justification to use a GTED threshold level of ≥1.5. The BPPD IRM Team notes that continuous planting of corn with Cry3Bb1 without rotation also leads to greater selection pressure. Hence, unexpected pest damage in such Cry3Bb1 fields with agronomic practices as described by Monsanto should be reason for concern and has a greater probability to be due to resistance evolution in that population. Therefore, the BPPD IRM Team maintains that a threshold level of 1.0 for a single toxin PIP is a more appropriate trigger to initiate adult collections.

1c) Monsanto stated that they had been working with Dr. Ostlie in Minnesota to understand the greater than expected root damage observed in Cry3Bb1 corn during the late corn growing season in 2009. The BPPD IRM Team interprets this to mean that Monsanto is unsure of the reasons for *Bt* failure and notes that this is an instance where collections should have occurred the following year (despite lower damage levels or population numbers). If growers 'bombed' adults in fall of 2009 or used IPM approaches to manage their fields, it should be expected that population densities and subsequent root damage were lower in 2010. However, resistance genes could still be in these populations (as discussed above), which is the reason why adult collections should be made once unexplained performance failures have occurred.

1d) Monsanto determined additional problem areas in South Dakota that were included into the sampling scheme for annual resistance monitoring in 2010 and proposes to use these to obtain baseline susceptibility data. These sites should not serve to obtain baseline data (as proposed by Monsanto) but rather measure shifts in CRW susceptibility to Cry3Bb1.

1e) Monsanto provided EPA with the number of performance inquiries (PI) for their Cry3Bb1 products in the Corn Belt from 2003 through 2010. The number of PIs ranged from 0 to over 100 per year during this time period. Monsanto reported that none of their follow-up investigations resulted, however, in finding resistant populations. With the exception of samples collected in 2007 and 2008, it is unclear to the BPPD IRM Team how many of the PI's reported in Table 4 resulted in adult collections and bioassays. This information should be provided for each state (each year) in addition to reporting the number of PIs. Furthermore, the BPPD IRM Team recommends that Monsanto include information about the degree of pest pressure and environmental conditions in those states with PIs (i.e. wet spring, etc).

2. THE BPPD IRM TEAM REVIEW OF 2009 CRW MONITORING REPORT

2a) After reviewing Monsanto's 2009 monitoring data for Cry3Bb1, the BPPD IRM Team concludes that the highest bioassay concentration tested (170 µg of Cry3Bb1/cm^2 of diet) does not appear to be adequate as a diagnostic assay for detecting resistance in WCRW populations. The EC/LC_{50} appears to detect shifts in WCRW susceptibility, but the bioassay results do not appear to be sensitive enough for discerning between populations with decreased susceptibility from those with actual resistance to the toxin. Monsanto should discuss with EPA whether the 'comparative weight' approach between *Bt* and non-*Bt* treatments for field populations could be used to develop a viable resistance detection level.

2b) Most mean LC_{50} values from 2009 population samples had to be extrapolated for the highest concentration because populations were no longer sensitive at this level of exposure. Monsanto and the BPPD IRM Team agree that LC_{50} results can be somewhat misleading when correlating larval survival to level of toxin susceptibility because larvae can survive on *Bt* for some time without feeding. A 3-day long assay may, therefore, capture less than the actual percent mortality due to Cry3Bb1 exposure. All field populations collected in 2009 had greater (mean) EC_{50} and LC_{50} values (measured and extrapolated) than those for the lab populations, in some instances by an order of magnitude.

2c) The only county from which population samples were obtained both in 2009 and 2008 was McLean (IL). In 2008, the EC_{50} and LC_{50} mean values were14.6 µg/cm^2 and 38.7µg/cm^2; in 2009, the EC_{50} and LC_{50} means were 37.75 µg /cm^2 and 585.06 µg/cm^2 (extrapolated value). This represents a two-fold and 100-fold increase for mean EC and LC-values, respectively in that location. Susceptibility in McLean County (IL) may have decreased during this time period, though the BPPD IRM team is not aware of any documented cases of field failures from this county.

2d) Monsanto tested WCRW populations from three states: Nebraska, Illinois, and Iowa. The BPPD IRM Team notes that these three states alone may not be sufficient and that Colorado, South Dakota (as proposed by Monsanto), Minnesota, and western Wisconsin may need to be included based on the registrant's reported Cry3Bb1 performance inquiries and discussions with corn entomologists (Gassmann et al., 2011b).

2e) Regarding the identified deficiency with respect to the terms and conditions of the registration (identified in BPPD 2010), Monsanto stated that Mexican and western corn rootworm were genetically similar and that therefore the WCRW IRM strategy should be appropriate for the MCRW. The BPPD IRM Team generally agrees that the assumptions may be appropriate; however, we note that there could still be differences in the specific dose profile that should be addressed.

3. THE BPPD IRM TEAM REVIEW OF UNEXPECTED CORN ROOTWORM DAMAGE IN CRY3Bb1 FIELDS AND CONCLUSIONS REGARDING SUSPECTED RESISTANCE TO CRY3Bb1

The BPPD IRM Team has been made aware of a number of cases of unexpected corn rootworm (CRW) damage to Cry3Bb1 fields. Some of these reports have been documented in the literature, while others are anecdotal. This section summarizes these reports and provides The BPPD IRM Team's review of them.

3a) The BPPD IRM Team reviewed Gassmann et al.'s (2011) report concluding that western corn rootworm (WCRW) had evolved resistance to Cry3Bb1 maize in certain parts of eastern Iowa. The BPPD IRM Team notes that populations tested in the assay originated from within problem fields where a certain proportion of adults can be assumed to have undergone development (egg hatching, immature development and emergence to adulthood). Some of the collected individuals may have also immigrated into these *Bt* fields from neighboring fields. If this was the case, then the resistance observed and described by Gassmann could be "diluted". More importantly, those native adults fed on and finished their development in Cry3Bb1 maize, were viable, and produced offspring that were also resistant. The subsequent progeny tested in greenhouses had significant and much greater survival on Cry3Bb1 maize than larvae that were fed on non-*Bt* maize and whose parental lines originated from control fields.

3b) In two published reports, Dr. Gray from the University of Illinois described severe performance issues with Monsanto's Cry3Bb1 corn in Henry and Whiteside County of northwestern Illinois (Gray 2011a) and LaSalle County of north-central Illinois (Gray 2011c). The problem fields had been planted to continuous corn for many years, Cry3Bb1 maize was planted to protect against WCRW damage, and similarities to nearby Iowa problem fields identified by Gassmann et al. (2011a) were noted. Between two to three nodes were missing on damaged root systems (Gray, 2011b); adults were numerous in these fields; lodging was prevalent; insect collections were made to determine whether resistance was the cause of Cry3Bb1 field failures. Dr. Gray proposed that growers who encountered field performance issues with CRW protected traits should consider rotating to a non-WCRW host, using CRW soil insecticide at planting and different CRW Plant-Incorporated Protectant (PIP), and/or planting a pyramided PIP expressing multiple Cry toxins in 2012, but that a long term IPM approach was necessary for successful CRW management.

3c) Over the course of 2011, The BPPD IRM Team has had multiple conference calls with scientists from academia and USDA-ARS to discuss performance of *Bt* corn in the Corn Belt. During a recent call on August 25, 2011, performance issues regarding Monsanto's Cry3Bb1 maize in Illinois were discussed (Gassmann et al. 2011b). The BPPD IRM Team was also informed that there were other severe efficacy issues for Monsanto's Cry3Bb1 trait in Nebraska (though spotty throughout the state), Minnesota (north-central and southern locations), and Iowa in 2011 (same areas as discussed in Gassmann et al. 2011a). Specifically in Minnesota, Dr. Ostlie reported that 2011 marked the third year that moderate to severe corn rootworm damage in Cry3Bb1 corn was observed and that the problem was becoming more widespread. In Nebraska, Drs. Siegfried, Meinke, and Hunt reported that the 2011 growing season marked the fourth year where moderate to severe rootworm damage in Monsanto's Cry3Bb1 corn was apparent. As in Illinois and Iowa, these problem fields were planted to continuous corn with Cry3Bb1 for several years in a row; no rotation with other crops occurred. It was unlikely that refuges were planted.

The BPPD IRM Team concludes that Cry3Bb1 resistance is suspected in at least some portions of Iowa, Illinois, Nebraska, and Minnesota, where "unexpected pest damage" reports originated. This conclusion is based on the following considerations:

- Western corn rootworm field populations in eastern Iowa have been documented with significant and increased survival on Cry3Bb1 maize compared to populations collected from non-problem fields and assayed on non-*Bt* maize (Gassmann et al. 2011a). The BPPD IRM TEAM recommends additional work be conducted on these populations to assess survival to adulthood, fecundity, and any other reproductive effects that could impact the populations' ability to survive in the field.

- Two recent reports published by the University of Illinois discussed severe Cry3Bb1 corn damage in northwestern and north-central Illinois during the 2011 corn growing season (Gray 2011a, 2011c). According to Dr. Gray's report, Henry County was among those that had severe Cry3Bb1 damage. Monitoring data supplied by Monsanto (see Table 6) from this county showed a six-fold increase in mean LC_{50} from 2007 to 2008 (the last year data were tabulated specifically from this county), though this result can not be directly correlated with any incidents of field damage.

- There are other reports of multiple and increased Cry3Bb1 performance failures in Nebraska and Minnesota (Gassmann et al. 2011b). Although Cry3Bb1 field failures throughout these four states (IA, IL, MN, and NE) of the Corn Belt are spotty, the frequency of such reports has apparently increased (through 2011).

- Resistance monitoring data for Cry3Bb1 have shown that field-collected populations (collected from Illinois, Iowa, and Nebraska) are generally less susceptible to the toxin than the laboratory reference colony. In 2009, every field-collected population had a higher EC_{50} and LC_{50} than the laboratory control, in some cases by an order of magnitude. This was also the case for populations collected in 2007 (see BPPD 2009). Monitoring data collected from 2005 to 2009 appear to show a large decrease in susceptibility over the time period (see Tables 5 and 6), however, much of the decrease in susceptibility is likely due to a change in toxin source in 2007.

Based on the conclusion of "suspected resistance", the BPPD IRM Team recommends that the appropriate remedial action plan be implemented for Cry3Bb1 corn in areas experiencing unexpected field damage. The remedial action plan includes the following steps for "suspected resistance" (BPPD 2010b):

- In cases of suspected resistance, the registrant will instruct growers to do one or more of the following:
    - During the present season, use conventional insecticides to control the adult stage of the suspected pest;
    - During the following season, use an alternative pest control method to deter establishment of potentially resistant insects.

The remedial action plan requires the use of alternate control measures to mitigate suspected resistance. However, the BPPD IRM Team notes that if Cry3Bb1 resistance has indeed developed (i.e. "confirmed resistance"), a 5% refuge for pyramids (i.e. SmartStax expressing Cry3Bb1 and Cry34/35 targeting corn rootworm) will be substantially less durable and could ultimately compromise the second unrelated toxin used to control the pest (i.e. in this case Cry34/35).

I. BACKGROUND

Corn rootworm (CRW) is among the most serious economic insect pests of corn in the United States (Levine & Oloumi-Sadeghi, 1991). Western corn rootworm (*Diabrotica virgifera virgifera*, WCRW) is found from Mexico to the U.S. Corn Belt and Canada and is widely distributed; Northern corn rootworm (*D. barberi*, NCRW) is found in the Midwest of the U.S and has a localized distribution. Together, they are the most prevalent *Diabrotica* pests in the U.S. The Mexican corn rootworm (*D. virgifera zea*, MCRW) has a distribution from Central America to the southern U.S. and is a sporadic problem in central Texas and southern Oklahoma. In 2003, the first transgenic (*Bt*) corn was registered to control CRW. Today, there are currently three registered *Bt* toxins available from different registrants to control target pest damage, all of which do not express a high dose against CRW. Simulation modeling predicts that pests are at greater risk of evolving resistance to *Bt* crops when toxins are less than high dose (Tabashnik et al., 2004).

In February 2003, the Agency approved Monsanto's registration of *Bacillus thuringiensis* (*Bt*) Cry3Bb1 protein and the genetic material (Vector ZMIR13L) necessary for its production. Corn expressing the Cry 3Bb1 protein, designated event MON 863 (YieldGard Rootworm, EPA Reg. No. 525-528) by Monsanto, was the first *Bt* corn product registered to protect crops against corn rootworm (CRW) species.

In December 2005, MON 88017 and MON 88017xMON810 were approved for registration. MON 88017xMON 810 expresses Cry3Bb1 and Cry1Ab and is targeted against corn rootworm (CRW) larvae (Cry3Bb) and European corn borer (ECB)/stalk boring lepidopteran larvae (Cry1Ab). The product was created by conventional breeding in which MON 88017 (EPA Reg. No. 524-LLR) was crossed with MON 810 (YieldGard, EPA Reg. No. 524-489). The Cry3Bb1 toxin expressed in MON 88017 is equivalent to that in MON 863. The Cry3Bb1 protein produced in MON 88017 and MON 863 is a variant of the wild-type Cry3Bb1 protein from *Bt* subspecies *kumamotoensis*, whereas the Cry1Ab toxin originated from *Bt* subspecies *kurstaki*. When compared by amino acid sequencing, the Cry3Bb1 protein expressed in MON 88017 has been reported to be 99.8% similar to the Cry3Bb1 protein expressed in MON 863. The primary difference between the hybrids is that MON 88017 also expresses a gene for resistance to glyphosate (Roundup) based herbicides.

MON 89034 x MON 88017 is a plant-incorporated protectant (PIP) that was registered for commercial use on June 10, 2008. MON 89034 expresses the *Bt*-derived insecticidal proteins Cry1A.105 and Cry2Ab2. The Cry1A.105 toxin is a "chimeric" protein containing domains I and II and the C-terminal from Cry1Ac and domain III from Cry1Fa (domain III). The Cry2Ab2 protein is exactly the same as that currently expressed in Monsanto's Bollgard II cotton.

SmartStax was conditionally registered by the Agency in July, 2009 with a 5% structured refuge for lepidoptera and corn rootworm. However, as a condition of registration, EPA requested further data be developed to address the uncertainties with dose (Cry3Bb1 and Cry34/35) and initial resistance allele frequency to support a 5% refuge for corn rootworm. EPA further recommended revised modeling to assess the risk of resistance evolution in corn rootworm incorporating these additional data.

This memorandum summarizes (Section I and II) and reviews (Section III) Monsanto's 2009 CRW resistance monitoring report for Cry3Bb1 corn and response to the Agency's recommendations for the proposed monitoring plan and questions regarding unexpected pest damage reports (BPPD 2010).

I. MONSANTO 2009 MONITORING REPORT (MRID 482080-01)

Objective:
To continue ongoing monitoring of susceptibility to *Bt* Cry3Bb1 protein in geographically distinct populations of WCRW by contrasting susceptibility of 2009 collections to a lab strain as well as data from previous years.

Methodology:
DM Crop Research Group (independent party collecting corn pest samples) collected adult CRW samples from 13 geographically distinct field populations in Nebraska, Iowa, and Illinois. Those population samples were sent to Custom BioProducts (an independent laboratory conducting bioassays for Monsanto since 2006), which maintained the insect collections and provided eggs and subsequent neonates for testing. Twelve samples of the thirteen samples yielded enough insects to perform bioassays with six replicates; one sample yielded enough individuals for four replicates. In addition, a (Monsanto) non-diapausing lab strain produced enough eggs for six control replicates.

The *E. coli* produced Cry3Bb1 used was a solution of 4.1 mg/ml supplied by Monsanto Company. This solution was diluted with 0.1% Titron-X 100 to obtain a series of concentrations of the Cry3Bb1 protein for bioassay. Based on the concentration of protein in the stock solution, the maximum concentration that could be used in the concentration-response assays was 170.8 µg/cm^2 of diet.

Neonate larvae were used in diet overlay bioassays and exposed to different Cry3Bb1 concentrations (10.7 µg/cm^2, 21.4 µg/cm^2, 42.7 µg/cm^2, 85.4 µg/cm^2, and 170.8 µg/cm^2). Thirteen µL of each dilution was applied to 12 individual wells and allowed to dry prior to larval introduction. Larvae were non-systematically selected and placed into wells of the tissue culture tray. After three days, mortality and survival were recorded.

The University of Nebraska performed statistical analyses using SAS and Probit Analysis to determine EC_{50}, EC_{95}, LC_{50}, and LC_{90} values as well as goodness of fit (*Chi*-Square probability) for each population tested.

Study Results and Discussion:
Monsanto reported that 2009 bioassay results indicated that WCRW populations throughout the Corn Belt remained susceptible to Cry3Bb1 as expressed in MON 88017 and MON 863.

Observed EC_{50} values ranged from 13.9 µg/cm^2 (Hamilton Co, NE) to 63.61 µg/cm^2 (Franklin Co, IA), representing a 4.5- fold difference in susceptibility. The Franklin County population sample had the greatest EC_{50} values of all 13 populations. The second highest EC_{50} value (41.33 µg/cm^2) was found in Sherman County (NE). With results from Franklin County excluded, the EC_{50} values of the field populations ranged from 13.90-41.11 µg/cm^2 and fell within the 95% confidence interval of the 2008 field populations (2.07-43.14 µg/cm^2). Monsanto concluded that although the EC_{50} value for the Franklin population was relatively high, that the bioassay results showed that the population was still susceptible to Cry3Bb1.

LC_{50} values were more variable than EC_{50} values. Higher LC-values however are an artifact of the assay system because larvae are able to survive without feeding. LC_{50} values of field derived populations ranged from 60.29-726.40 µg of Cry3Bb1/cm^2 (excluding populations where the statistical program failed to produce probit results).

Table 1. Susceptibility of Western Corn Rootworm Populations to Cry3Bb1 measured by EC/LC_{50}s (2009 Data)

Population	# of Reps	EC_{50} (95% C.I.) (µg of Cry3Bb1/cm^2)	LC_{50} (95% C.I.) (µg of Cry3Bb1/cm^2)
Franklin Co, NE	6	63.61 *(40.75-113.01)*	*
McLean Co, IL	6	37.75 *(30.19-46.89)*	585.06 (*216.57-30312*)
Clay Co, IL	6	14.12 *(8.97-19.12)*	*
Champaign Co, IL	6	39.88 *(37.16-42.77)*	579.18 (*246.06-7377*)
Sherman Co, NE	6	41.33 *(30.19-56.38)*	314.32 *9169.59-15850*
Story Co, IA	6	28.88 *(22.32-36.20)*	726.40 *(231.61-265519)*
Scott Co, IA	6	16.70 *(11.52-21.82)*	*
Bureau Co, IL	6	18.29 *(11.17-25.59)*	277.53 *(146.01-1495)*
Howard Co, NE	6	40.61 *(25.85-62.82)*	*
Hamilton Co, NE	4	13.90 *(6.79-20.76)*	60.29 *(36.65-119.73)*
Iroquois Co, NE	6	26.14 *(17.26-36.57)*	*
Ford Co, NE	6	18.55 *(13.04-24.07)*	188.14 *(108.04-651.35)*
Peoria Co, IL	6	21.71 *(15.47-28.24)*	194.32 *(113.44-627.48)*
Laboratory Control	6	9.25 *(3.22-15.22)*	51.59 *(33.98-82.79)*

*POLO failed to produce probit results for these collections

Monsanto tested the diagnostic concentration of 170.8 µg/cm^2 for the second consecutive field season and determined that although 100% mortality was not obtained, survivors at this concentration exhibited severe stunting (as measured by average body mass). The reduction in average mass ranged from ⅛ to ¼ (0.03-0.06 mg) of the control groups average mass (0.25 mg). For example, survivors of the Franklin County population had an average body mass of 0.06 mg, which was comparable to other field populations and significantly less than the mass of all field populations at the control dose.

Table 2. Mean mortality and average mass of survivors from field populations at the highest Cry3Bb1 concentration

Population	% Mortality Across Replicates	Average Mass of Survivors (*mg*) Across Replicates	Maximum Mass Across Replicates (*mg*)
Franklin Co, NE	50.00	0.06	0.09
McLean Co, IL	43.06	0.05	0.06
Clay Co, IL	52.78	0.03	0.05
Champaign Co, IL	34.72	0.05	0.08
Sherman Co, NE	45.83	0.05	0.08
Story Co, IA	40.28	0.05	0.08
Scott Co, IA	48.61	0.04	0.06
Bureau Co, IL	48.61	0.03	0.05
Howard Co, NE	33.33	0.05	0.08
Hamilton Co, NE	66.67	0.03	0.05
Iroquois Co, NE	58.33	0.04	0.06
Ford Co, NE	48.61	0.06	0.08
Peoria Co, IL	52.78	0.04	0.07
Ranges	33.33-66.67	0.03-0.06	0.05-0.09
Laboratory Colony	68.06	0.03	0.04

Table modified from Monsanto's original submission (MRID 482080-01)

Table 3. Control mean mortality and mass of survivors of field populations on non-*Bt* containing diet

Control - Field Populations	Mean % Mortality Across Replicates	Average mass of Survivors (*mg*) Across Replicates	Maximum Mass Across Replicates (*mg*)
All sites	9.24	0.25	0.30

Table extracted from Monsanto's original submission (MRID 482080-01)

II. MONSANTO'S RESPONSE TO THE BPPD IRM TEAM'S REVIEW OF THE APPLICANT'S 2008 MONITORING PLAN

Monsanto stated that it utilized the root damage threshold level of 1.5 (on the 0-3 Node Injury Scale, NIS) in *Bt* fields with greater than expected damage (GTED) and 50% of the sampled plants with >1.5 to determine if additional actions were needed. High rootworm pressure was typically the result of continuous-corn plantings that built up CRW population densities over time or presence of diapausing variant of the NCRW or soybean variant of WCRW (in corn-soy-corn crop rotations). Additionally, Monsanto proposed that a threshold of at least 50% of the sampled plants with >1.0 node injury would be better for a pyramided product such as SmartStax.

Monsanto investigated all product performance incidents using a standardized process for collection of agronomic and field information. After the growers informed local sales seed representatives (LSSR), the sale reps investigated the claim and collected information as well as representative root samples from the field (and adjacent refuge field if present). At this point, if the node injury damage was less than 1.5, Monsanto stated that the LSSR worked to understand the damage and resolved the incident with the growers. If the node injury damage was greater than 1.5, seed representatives worked with the technology development

representative to assess the situation and verified if average root damage was greater than 1.5 and 50% of the roots were damaged in the field of concern. After this assessment, they decided whether the results met the criteria to trigger CRW beetle sampling. Sampling would occur at this time or, if adults were no longer present, the field would be monitored the following year for root damage. If the root damage rating exceeded 1.5 at that time, adults would be collected in the problem fields.

Monsanto reported that in 2007 and 2008, five and eight samples of adult beetles, respectively were collected from fields with excess damage (>1.5) and sent to Custom Bio-Products for bioassays. No populations were collected from fields with performance issues in 2009 due to late notifications. None of populations that stemmed from individuals collected in 2007 and 2008 showed a significant decrease in susceptibility relative to historical results and routine monitoring conducted in those years (no actual bioassay data were submitted in this report). For example, in 2008 Monsanto responded to 32 individual field performance issues (see Table 4). Of those only three had node injury scores >1.5, and in only two fields beetles could be collected. Additionally, Monsanto collected CRW samples in Colorado, Illinois, Kansas, and Nebraska where historical pest pressure and root damage were high. Eight populations were reared in the lab, and standard diet bioassays were conducted with neonates. The conclusions from the assays were that all eight populations remained susceptible to Cry3Bb1 when compared to populations assayed in the routine monitoring. Monsanto reported that the reasons for the 'greater than expected damage' in Cry3Bb1 corn were uncertain.

Table 4. Number of performance inquiries by state and year for MON 88017 and MON 863 products

State	2003	2004	2005	2006	2007	2008	2009	2010
CO	0	0	0	9	3	12	23	13
IL	0	13	4	13	15	0	3	0
IN	0	0	0	0	0	0	0	0
IA	0	0	2	11	21	3	23	13
KS	0	3	3	2	13	4	8	7
MN	0	0	2	0	0	1	15	3
NE	0	5	12	58	46	11	30	47
OH	0	0	0	0	1	0	0	0
SD	0	0	0	0	3	0	0	9
WI	0	0	4	0	0	0	4	1
Other	0	0	5	1	11	1	1	5
Total CRW related PIs[1]	0	21	32	94	103	32	107	98
MON CRW *Bt* Acres *	354	1586	3271	9227	19869	28949	30631	28747
MON 863 Acres *	354	1586	3271	9227	18385	5899	2018	800
MON 88017 Acres *	0	0	0	0	1484	23050	28613	27947

Table extracted from Monsanto's submission (MRID 484368-01)
*calculations based on 2.6 acres per unit of seed sold; SmartStax is not included in the 2010 statistics
[1] PIs = performance inquiries

The BPPD IRM Team (BPPD 2010) raised concerns with Monsanto's protracted response to unexpected damage which could take up to two seasons before the registrant responds to a potential resistance event (according to the BPPD IRM Team's internal analysis of Monsanto's reports). Monsanto responded that their policy as good stewards of MON88017 and MON 863 was to respond to every case of GTED (greater than expected damage) and collect adult insects whenever possible. When Monsanto is contacted too late in the

season to collect adults, the area of concern is monitored the following growing season, and if GTED occurs again, adults will be collected.

The BPPD IRM Team (BPPD 2010) also disagreed with Monsanto on their plan to collect (potentially resistant) insects >1/2 mile away from problem fields. Monsanto clarified that once the GTED had been established in a field that Monsanto representatives would collect 500-1000 individuals from the neighboring area within a 1 to 2 mile radius of the problem site.

In 2009, Dr. Ostlie reported Cry3Bb1 field failures in northern Minnesota and both NCRW and WCRW adults were present in abundant numbers. The BPPD IRM Team (BPPD 2010) requested that Monsanto provide a detailed description about follow up activities in those areas of Minnesota. Monsanto reported that in most cases the root damage rating did not exceed 1.5 in 50% of the Cry3Bb1 plants, hence no further action was pursued. In the Cry3Bb1 fields with GTED, no adult collections were made in 2009 because the reports were received too late, and the same damage was not observed the following year. Monsanto has been working with Dr. Ostlie in Minnesota to better understand the reports of GTED. However, Monsanto responded that in 2010 they followed up on performance inquiries in two affected southern Minnesota fields that were near a site that had reported problems in 2009. No adults were collected because no GTED was observed. Additionally, two populations in an affected area were sampled in South Dakota. Those were near 2009 Minnesota problem sites and were included to initiate additional monitoring in the area and to establish a baseline in the area.

The BPPD IRM Team (BPPD 2010) recommended that Monsanto include MN and WI into their ABSTC sampling region 2 beginning with the 2009 CRW monitoring collections. In addition, we also recommend that Monsanto obtain samples from the eastern IA region where Gassmann et al. (2011) reported Cry3Bb1 durability issues. Monsanto stated that beginning in 2010, ABSTC included additional population samples from areas in Iowa and Minnesota, though none were collected in 2009 (beetle densities were, however, low making it impossible to successfully collect in most regions). Additionally, Monsanto included three new locations in northeastern IA and two new sites in SD from where beetles were collected during the 2010 growing season (results to be submitted to Agency in 2011).

The BPPD IRM Team (BPPD 2010) further requested that Monsanto report on the progress of developing assays for northern corn rootworm before March 31, 2011. The BPPD IRM Team also recommended that Monsanto take up monitoring and the development of baseline susceptibility data for NCRW as outlined in the terms and conditions of the registration; join with other registrants and develop an ABSTC style unified sampling strategy for CRW based on high risk and adoption of PIPs. Monsanto responded that efforts were underway through ABSTC to review the available knowledge on NCRW rearing and bioassay, and to investigate any promising new developments but that presently no labs were successfully rearing and testing the northern corn rootworm; companies joined and established a unified sampling strategy for WCRW (through ABSTC) and had established sampling regions.

The BPPD IRM Team (BPPD 2010) had identified a deficiency in Monsanto's response to the terms and conditions of registration in that Monsanto had not reported whether IRM strategies designed for WCRW and NCRW were appropriate for the Mexican corn rootworm. Monsanto reported (MRID 484368-01) that WCRW and MCRW appear to be genetically similar (Szalanski et al. 1999).

III. BPPD IRM TEAM REVIEW AND CONCLUSIONS

1. 2009 MONITORING DATA

The BPPD IRM Team reviewed Monsanto's LC_{50} values for the 2009 CRW monitoring season (highest concentration of 170 µg/cm^2 was used) and notes that most mean values were extrapolations from lower concentrations because populations were no longer sensitive at this level of exposure. Monsanto and the BPPD IRM Team agree that LC_{50} results can be somewhat misleading when correlating larval survival to level of toxin susceptibility because larvae can survive on *Bt* for some time without feeding. A 3-day long assay may, therefore, capture less than the actual percent mortality due to Cry3Bb1 exposure.

When looking at the historical EC_{50} and LC_{50} ranges for field populations, it appears that there is a visible downward trend in susceptibility since 2005 (Table 5). Much of the apparent reduced susceptibility occurred between 2006 and 2007 and is likely due to a change in toxin source to less active version of Cry3Bb1 (see BPPD 2009). Because of this, data collected prior to 2007 cannot be directly compared to the more recent results. Nevertheless, the results show that field-collected populations have been generally less susceptible to Cry3Bb1 than the laboratory control colonies. For example, all of the field populations collected in 2009 had greater (mean) EC_{50} and LC_{50} values (measured and extrapolated) than those for the lab populations, in some instances by an order of magnitude (see Table 1). This was also the case in the 2007 collections (though not in 2008).

One population from Franklin County, Nebraska had a mean EC_{50} value that fell outside of the 95% confidence interval of the 2008 field populations (2.07-43.14 µg/cm^2). Monsanto reported that there was no reason for concern because the mean weight of the sample was approximately one third that of the mean weight of the control population (see Tables 2 and 3). It is unclear from the submitted report whether these individuals were tracked to potential adulthood after the assays were terminated or whether Monsanto decided that the decrease in weight meant that individuals were essentially unfit to survive. The BPPD IRM Team recommends that Monsanto clarify this point and concludes that the highest concentration tested (170 µg of Cry3Bb1/cm^2 of diet) does not appear to be adequate for monitoring as a diagnostic con resistant in WCRW populations. The EC/LC_{50} appears to detect shifts in WCRW susceptibility, *but the bioassay results do not appear to be sensitive enough for discerning between populations with decreased susceptibility from those with actual resistance to the toxin.* It is recommended that Monsanto discuss with the Agency whether the 'comparative weight' approach between *Bt* and non-*Bt* treatments for field populations could be used to develop a "viable" resistance detection level.

The only county from which population samples were obtained both in 2009 and 2008 was McLean (IL). In 2008, the EC_{50} and LC_{50} means were14.6 µg/cm^2 and 38.7 µg/cm^2, and their minimum and maximum values ranged from 7.5 to 21.4 µg /cm^2 and 19.1 to 63.6 µg /cm^2, respectively; in 2009, the EC_{50} and LC_{50} mean values were 37.75 µg /cm^2 and 585.06 µg/cm^2 (extrapolated value), and their minimum and maximum values ranged from 30.19 µg/cm^2 to 46.89 µg/cm^2 and 216 to 39312 µg/cm^2, respectively. The 2009 values were a two-fold and >100-fold increase for mean EC and LC-values, respectively, compared to 2008 values. The maximum values of the ranges of 2008 increased by two- and >60-fold for estimated EC_{50} and extrapolated LC_{50}-values respectively. Most mean LC values in 2009 could not be measured at the highest concentration and had to be extrapolated based on treatments with lower concentrations.

Monsanto tested CRW populations from three states: Nebraska, Illinois, and Iowa. The BPPD IRM Team notes that these three states alone may not be sufficient and that Colorado, Minnesota, and western Wisconsin may

need to be included based on Monsanto's information (Table 4) regarding Cry3Bb1 performance issues and other discussions with corn entomologists (Gassmann et al., 2011b).

Regarding the identified deficiency with respect to the terms and conditions of the registration (BPPD 2010), Monsanto stated that Mexican and western corn rootworm were genetically similar and that therefore the WCRW IRM strategy should be appropriate for the MCRW. The BPPD IRM Team generally agrees that the assumptions may be appropriate. We note, however, that there could still be differences in the specific dose profile that should be addressed.

Table 5. Susceptibility results across years for Cry3Bb1

Year	EC_{50} (µg/cm^2)		LC_{50} (µg/cm^2)	
	field collected	**laboratory**	**field collected**	**laboratory**
2005	0.28-2.64	N/A	0.31-4.59	N/A
2006	0.64-1.88	N/A	1.43-22.22	N/A
2007	14.20-33.46	12.91	50.18-289.25	22.29
2008	7.3-30.4	21.8	24.5-335	87.9
2009	13.9-63.61	9.25	60.29-726.40	51.59

Table generated from data submitted by Monsanto.

2. MONSANTO'S MONITORING PLAN AND FURTHER RESPONSES TO BPPD (2010)

After reviewing Monsanto's monitoring plan, the BPPD IRM Team concludes that the applicant's current resistance monitoring program is ineffective and likely to miss early resistance events. This conclusion was based on the following observations:

a. The registrant proposed to collect insects from problem fields within 1-2 miles from neighboring sites of failed fields. The BPPD IRM Team believes that this proposal is insufficient if the goal is to determine whether *Bt* failure is due to resistance of CRW population. Since the majority of adult corn rootworm may not disperse long distances, the greatest probability of capture of resistant genotypes should be in the problem fields, possibly in adjacent fields, but less likely in fields 2 miles away during that particular year. To collect in neighboring sites is reasonable after infield collections have occurred or during the following year because of possible adult movement. It is imperative that the language for this crucial procedure is unambiguous and clear and that Monsanto and EPA come to a timely agreement on the method of collecting adult CRW in problem fields. If the resistance monitoring program is to be meaningful, a more proactive, effective approach needs to be adopted.

b. The BPPD IRM Team concludes that Monsanto's GTED threshold of 1.5 (on the NIS) for a single toxin PIP with non-high dose expression is generally too high. The BPPD IRM Team stated in the 2010 review that injury ratings of 1.0 should trigger investigations. Ideally, a comparison should occur between performance of *Bt* and nearby refuge corn; however, this may not be an option if growers have not complied with refuge requirements. In such non-compliant cases, the probability that severe root damage is due to resistance should be greater and, therefore, the BPPD IRM Team considers threshold level of 1.0 for a single toxin PIP a more defensible (conservative) trigger to initiate adult collections. During years with great pest pressure (not associated with agronomic practices such as growing

continuous corn), EPA notes that it may be reasonable to deviate from the 1.0 threshold and use 1.5 (based on a graded scale where *Bt* damage is related to adult density and damage to refuge corn). The BPPD IRM Team concludes that for SmartStax products (i.e. SSX RIB), a threshold of 1.0 (as proposed by Monsanto) is not conservative enough; this was already discussed in BPPD (2010). The pyramid has greater activity against CRW and should, therefore, have a lower threshold (such as 0.5) to trigger beetle collections. Additionally, in a seed blend it will be inherently more difficult to detect *Bt* performance issues, therefore, it may not be realistic to use the following threshold "50% of the plants must show greater than 0.5 (NIS) damage" before an investigation occurs. The BPPD IRM Team proposes that any clearly visible sign of damage exceeding the threshold level (0.5) in a seed blend (irrespective of the percentage of *Bt* plant damage in that field) trigger adult collections.

c. If Monsanto is unable to collect adults from problem fields because they were contacted too late by seed dealers, then it is important that adult sampling occurs in the problem areas the following season irrespective of pest pressure and damage in the old problem fields. If growers sprayed their fields after they observed high densities of adults during a problem year or used other methods of control, then the pest pressure and subsequent *Bt* damage should be less severe the following year. If Monsanto does not collect in such problem areas during the following season because of "below threshold damage" in *Bt* fields (1.0 for single PIPs and 0.5 for SSX), then they will miss early resistance events in those areas. If there is resistance but low pest pressure, damage can be expected to be lower than in cases where there is high pest pressure coupled with resistant individuals in the population.

Monsanto reported that high rootworm pressure was typically the result of continuous-corn plantings that built up CRW population densities over time and used this reasoning as a justification to use a GTED threshold level of 1.5. The BPPD IRM Team notes that continuous planting of corn with Cry3Bb1 without rotation also leads to greater selection pressure. Hence, unexpected pest damage in such Cry3Bb1 fields with agronomic practices as described by Monsanto should be reason for concern and has a greater probability to be due to resistance evolution in that population. Therefore, the BPPD IRM Team maintains that a threshold level of 1.0 for a single toxin PIP is a more appropriate trigger to initiate adult collections.

Monsanto stated that they had been working with Dr. Ostlie in MN to understand the greater than expected root damage observed in Cry3Bb1 corn during the late corn growing season in 2009. The BPPD IRM Team interprets this to mean that Monsanto is unsure of the reasons for *Bt* failure and notes that this is an example of instance where collections should have occurred the following year (despite lower damage levels or population numbers). If growers 'bombed' adults in fall 2009 or used IPM approaches to manage their fields, it should be expected that population densities and subsequent root damage were lower in 2010. However, resistance genes could still be in these populations, which is the reason why adult collections should be made once unexplained performance failures have occurred.

Monsanto determined additional problem areas in South Dakota that were included into the sampling scheme for annual resistance monitoring in 2010 and proposes to use these to obtain baseline susceptibility data. These sites should not serve to obtain baseline data but rather to measure shifts in CRW susceptibility to Cry3Bb1.

Monsanto provided EPA with the number of performance inquiries for their Cry3Bb1 products in the Corn Belt from 2003 through 2010. The number of PIs ranged from 0 to over 100 per year during this time period. Monsanto reported that none of their follow up investigations resulted, however, in finding resistant populations. With the exception of samples collected in 2007 and 2008, it is unclear to the BPPD IRM Team how many of the PI's reported in Table 4 resulted in adult collections and bioassays. This information should be provided for each state (each year) in addition to reporting the number of PIs. Furthermore, the BPPD IRM Team recommends that Monsanto include information about the degree of pest pressure and environmental

conditions in those states with PIs (*i.e.* wet spring, etc.).

The BPPD IRM Team notes that Dr. Gray provided information that supports Monsanto's reporting of having had no Cry3Bb1 performance inquiries during the 2010 corn growing season in Illinois. Dr. Gray stated that damage to Cry3Bb1 maize was mild and that generally adult population levels were low throughout the state. A wet spring was likely responsible for these conditions (Gray (2011b), personal communication).

The BPPD IRM Team recommends that Monsanto provide an update on the development assays, resistance monitoring, and the development of baseline susceptibility data for northern corn rootworm with the next monitoring report.

3. THE BPPD IRM TEAM'S REVIEW OF UNEXPECTED CORN ROOTWORM DAMAGE IN CRY3Bb1 FIELDS AND CONCLUSIONS REGARDING SUSPECTED RESISTANCE TO CRY3Bb1

The BPPD IRM Team has been made aware of a number of cases of unexpected corn rootworm damage to Cry3Bb1 fields. Some of these reports have been documented in the literature, while others are anecdotal. This section summarizes these reports and contains the BPPD IRM Team's analysis regarding "suspected resistance" to Cry3Bb1 corn.

During a presentation, Dr. Gassmann alerted the scientific community at the Entomological Society of America (ESA) meeting in San Diego (2010) that western corn rootworm had evolved resistance to Monsanto's Cry3Bb1 corn in certain Iowa locations. The official report was subsequently published by Gassmann et al. (2011a). The following is the BPPD IRM Team's summary of the publication.

In 2009, Dr. Gassmann was alerted to severe feeding damage in four Cry3Bb1 fields in eastern Iowa. At that time, adults were collected in reported problem fields as well as in five non-*Bt* control fields throughout the state. Rootworm damage was assessed in *Bt* fields but not the control fields. Interviews with farmers revealed that growers had planted Cry3Bb1 maize for at least three continuous seasons in problem fields. No information about compliance was gathered although the authors speculated that non-compliance may have contributed to the observed effects. The adults collected during 2009 were held in separate cages and allowed to mate at the Iowa State University. The resulting eggs/neonates were examined using a 17-day long seedling assay on plants that were grown in the greenhouse. The assay used two different *Bt* expressing seedlings (DeKalb Cry3Bb1 and Mycogen Cry34/35) and DeKalb and Mycogen non-*Bt* varieties as controls. Survival of larvae from Cry3Bb1 problem fields was three times higher and significantly greater than survival of larvae from control fields reared on non-*Bt* expressing plants. Gassmann et al.'s preliminary analysis suggested that WCRW resistance was incomplete, that problem fields contained a mixture of resistant and susceptible genotypes, or that a combination of these conditions was present. Survival of control larvae, however, was significantly lower on Cry3Bb1 maize compared to non-*Bt* expressing plants. Offspring of adults from problem fields had significantly lower survival on Cry34/35 compared to non-*Bt* maize, and no difference was detected between survival on Cry34/35 of offspring from problem and control fields.

A significant positive correlation was detected between the number of years growers chose to plant Cry3Bb1 maize and the survival on Cry3Bb1 maize of insects from problem fields. The researchers concluded that WCRW was evolving resistance in some areas of Iowa and that the following factors likely supported the evolution of resistance: non-high dose of Cry3Bb1 corn resistance is non-recessive and insufficient insects from refuge populations. Gassmann et al. (2011a) suggested that there was a direct corollary between their findings in the problem fields (Cry3Bb1 grown for at least three consecutive years) to the lab experiment by Meihls et al. (2008) where WCRW resistance evolved after just three generations of selection on Cry3B maize.

During a conversation with the BPPD IRM Team, Dr. Gassmann explained that damage in the same 2009 Iowa problem fields did not occur in 2010 and 2011 because growers typically change their practice to avoid further field failures the following year (i.e. adult bombing, planting other PIPs, other IPM methods). He noted though that similar Cry3Bb1 field damage occurred just a few miles from where the original field failures were reported (Gassmann 2011, personal communication).

Additionally, the BPPD IRM Team has been made aware of severe Cry3Bb1 field damage in western Illinois near the sites of the field failures reported by Gassmann (Gray 2011b, personal communication). A report published by the University of Illinois described severe performance issues with Monsanto's Cry3Bb1 corn in Henry and Whiteside County (Gray 2011a). The problem fields had been planted to continuous corn for many years, Cry3Bb1 maize was planted to protect against WCRW damage, and similarities to nearby Iowa problem fields identified in Gassmann et al. (2011a) were noted. Between two to three nodes were missing on damaged root systems (Gassmann et al. 2011b); adults were numerous in these fields; lodging was prevalent; insect collections were made to determine whether resistance was the cause of Cry3Bb1 field failures. In the report it was proposed that growers who encountered field -performance issues with CRW protected traits should consider rotating to a non-WCRW host, using CRW soil insecticide at planting and different CRW PIP, and/or planting a pyramided PIP expressing multiple Cry toxins in 2012 but that a long term IPM approach was necessary for successful CRW management (Gray 2011a). On September 23, 2011, Dr. Gray published another report describing more Cry3Bb1 field failures (several nodes were completely missing from the roots) in north-central Illinois (i.e. LaSalle County). The affected fields shared that they had been grown to Monsanto's Cry3Bb1 corn for many successive years without crop rotation (Gray 2011c).

Over the course of 2011, the BPPD IRM Team has had multiple conference calls with scientists from academia and USDA-ARS to discuss performance of *Bt* corn in the Corn Belt. During a recent call on August 25, 2011, the BPPD IRM Team discussed with these corn entomologists the performance issues regarding Monsanto's Cry3Bb1 maize in Illinois (Gassmann et al. 2011b). At that time, the BPPD IRM Team was informed that there were other severe performance issues for Monsanto's Cry3Bb1 trait in Nebraska (spotty though throughout the state), Minnesota (north-central and southern locations), and Iowa in 2011 (some areas as discussed in Gassmann et al. 2011a). Specifically in Minnesota, Dr. Ostlie reported that 2011 marked the third year that moderate to severe corn rootworm damage in Cry3Bb1 corn was observed and that the problem was becoming more widespread. In Nebraska, Drs. Siegfried, Meinke, and Hunt reported that the 2011 growing season marked the fourth year where moderate to severe rootworm damage in Monsanto's Cry3Bb1 corn was apparent. As in Illinois and Iowa, these problem fields were planted to continuous corn with Cry3Bb1 for several years in a row (cattle farmers who need corn for feed or growers who planted corn for ethanol production); no rotation with other crops occurred. It was unlikely that refuges were planted. Gravid females were collected from these sites and their offspring will be analyzed. According to these researchers, many growers still plant this trait despite the incidences of rootworm damage. When growers encounter great numbers of adult corn rootworm, they convert back to adult beetle "bombing" so that Cry3Bb1 can be planted again the following season.

The BPPD IRM Team reviewed Gassmann et al.'s report (2011a) and notes that population samples tested in the assay originated from within problem fields where a certain proportion can be assumed to have undergone development (egg hatching, immature development and emergence to adulthood). Some of the collected individuals may have also immigrated into these *Bt* fields from neighboring fields. If this was the case, then the resistance observed and described by Gassmann could be "diluted". More importantly though, those native adults fed on and finished their development in Cry3Bb1 maize, were viable, and produced offspring that were also resistant. The progeny tested in greenhouses had significant and much greater survival on Cry3Bb1 maize than larvae fed on non-*Bt* maize and whose parental lines originated from control fields. The proximity of western Illinois fields with failed Cry3Bb1 maize is disconcerting. The BPPD IRM Team concludes that

Gassmann et al. demonstrated that there is increased WCRW tolerance to Cry3Bb1 in some eastern Iowa field populations, and that the observed response had a heritable component. The researchers did not analyze or report if the progeny survived to adulthood. To fully characterize these populations the BPPD IRM Team recommends additional work be conducted on these populations to assess survival to adulthood, fecundity, and any other reproductive effects that could impact the populations' ability to survive in the field.

Overall, the BPPD IRM Team concludes that Cry3Bb1 resistance is suspected in at least some portions of the four states in which these reports originated (IA, IL, MN, and NE). This conclusion is based on the following weight of evidence:

- Gassmann et al. (2011a) showed that WCRW field populations in eastern Iowa had significant and increased survival on Cry3Bb1 maize compared to populations collected from non-problem fields and assayed on non-*Bt* maize. The researchers did not report, however, whether the progeny survived to adulthood.

- Two recent reports published by the University of Illinois discussed severe Cry3Bb1 corn damage in northwestern and north-central Illinois during the 2011 corn growing season (Gray 2011a, 2011c). According to Dr. Gray's report, Henry County was among those that had severe Cry3Bb1 damage. Monitoring data supplied by Monsanto (see Table 6) from this county showed a six-fold increase in mean LC_{50} from 2007 to 2008 (the last year data were tabulated specifically from this county), though this result can not be directly correlated with any incidents of field damage.

- There are other reports of multiple and increased Cry3Bb1 performance failures in Nebraska and Minnesota (Gassmann et al. 2011b). Although Cry3Bb1 field failures throughout four states of the Corn Belt are spotty (IA, IL, MN and NE,), the frequency of such reports has apparently increased (through the 2011 season).

- Resistance monitoring data for Cry3Bb1 have shown that field-collected populations (collected from Illinois, Iowa, and Nebraska) are generally less susceptible to the toxin the laboratory reference colony. In 2009, every field-collected population had a higher EC_{50} and LC_{50} than the laboratory control, in some cases by an order of magnitude. This was also the case for populations collected in 2007 (see BPPD 2009). Monitoring data collected from 2005 to 2009 appeared to show a large decrease in susceptibility over the time period (see Tables 5 and 6), however, the great drop off in susceptibility between 2006 and 2007 was likely due to a change in toxin source.

Table 6. Susceptibility in Three Counties across the Corn Belt as Measured by EC_{50} and LC_{50} Values (2005-2008 Data)

Population	Mean EC_{50} ($\mu g/cm^2$)	Mean LC_{50} ($\mu g/cm^2$)
Scott County, IA (2005)	0.3	0.5
Scott County, IA (2006)	1.7	6.6
Scott County, IA (2007)	15.5	63.8
Scott County, IA (2008)	25.8	40.0
Henry County, IL (2005)	1.9	3.2
Henry County, IL (2006)	1.9	5.6
Henry County, IL (2007)	16.2	50.2
Henry County, IL (2008)	14.8	300.9
Seward County, NE (2005)	2.6	3.3
Seward County, NE (2006)	1.3	9.3
Seward County, NE (2007)	14.2	64.2
Seward County, NE (2008)	9.4	335.0
Monsanto Reference Strain (2007)	***12.9***	**22.3**
Monsanto Reference Strain (2008)	***21.8***	**87.9**

Table generated from data submitted by Monsanto.

Based on the conclusion of "suspected resistance", the BPPD IRM Team recommends that the appropriate remedial action plan be implemented for Cry3Bb1 corn in areas experiencing unexpected field damage. The remedial action plan includes the following steps for "suspected resistance":

- In cases of suspected resistance, the registrant will instruct growers to do one or more of the following:
    - During the present season, use conventional insecticides to control the adult stage of the suspected pest;
    - During the following season, use an alternative pest control method to deter establishment of potentially resistant insects.

The remedial action plan requires the use of alternate control measures to mitigate suspected resistance. However, the BPPD IRM Team notes that if Cry3Bb1 resistance has indeed developed (i.e. "confirmed resistance"), a 5% refuge for pyramids (i.e. SmartStax expressing Cry3Bb1 and Cry34/35 targeting corn rootworm) will be substantially less durable and could ultimately compromise the second unrelated toxin used to control the pest (i.e. in this case Cry34/35).

IV. REFERENCES

BPPD. 2009. Review of Monsanto's 2005-2007 Corn Rootworm Monitoring Report and Revised CRW Resistance Monitoring Plan for MON 88017, MON 88017 x MON 810, and MON 89034 x MON 88017 (EPA Reg. Nos. 524-528, 524-545, 524-551, 524-552, and 524-576). Memo from JC Martinez to Mike Mendelsohn dated August 24, 2009.

BPPD. 2010. Review of Monsanto's 2008 Corn Rootworm Monitoring Report and Revised CRW Resistance Monitoring Plan for MON 88017, Mon 88017 x MON 810, MON 863, MON 863 x MON810, MON 89034 c TC1507 x MON 88017 x DAS-59122-7, and MON 89034 x MON 88017. Memo from JC Martinez to Mike Mendelsohn dated June 30, 2010.

Gassmann AJ, JL Petzold-Maxwell, RS Keweshan, and MW Dunbar. 2010. Screening field-populations of western corn rootworm for susceptibility to *Bt* corn. Presentation at the Entomological Society of America in San Diego, December 2010.

Gassmann AJ, JL Petzold-Maxwell, RS Keweshan, and MW Dunbar. 2011a. Field-evolved resistance to *Bt* maize by western corn rootworm. PLOS one, Vol. 6 (7): 1-7

Gassmann A, M Gray, R Hellmich, T Hunt, L Meinke, B Siegfried, and K Ostlie. Personal communication with JC Martinez and A Reynolds on August 25, 2011b.

A Gassmann, personal communication with JC Martinez on September 14th, 2011.

M Gray. Severe root damage to *Bt* corn observed in northwestern Illinois. The Bulletin, No. 20, August 26, 2011a. http://bulletin.ipm.illinois.edu/article.php?id=1555

M Gray, email communication with JC Martinez on August 18, 2011b.

M Gray. Additional reports of severe rootworm damage to *Bt* corn received: questions and answers. http://bulletin.ipm.illinois.edu/article.php?id=1569 . The Bulletin, No. 22, September 23, 2011c.

G Head. 2011. Conditions of registration for MON 88017 and MON 89034 x MON 88017, and response to EPA's request for additional information on Monsanto's resistance corn rootworm monitoring program. Monsanto unpublished report to the Agency (MRID 484368-01).

B Lang. 2010. 2009 season monitoring for the susceptibility of neonate western corn rootworm larvae to the *Bacillus thuringiensis* Cry3Bb1 protein. Monsanto unpublished report to the Agency (MRID 482080-01).

Meihls LN, ML Higdon, B Siegfried, NJ Miller, TW Sappington, MR Ellersieck, TA Spencer, and BE Hibbard. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. PNAS, Vol. 105(49): 19177-19182

US EPA. 2010. Biopesticide Registration Action Document for *Bacillus thuringiensis* Cry3Bb1 Protein and the Genetic Material Necessary for Its Production (Vector PV-ZMIR13L) in MON 863 Corn (OECD Unique Identifier: MON-ØØ863-5). http://www.epa.gov/oppbppd1/biopesticides/pips/cry3bb1-brad.pdf