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

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

August 24, 2007

MEMORANDUM

SUBJECT: Review of Dow AgroScience's (and Pioneer HiBred's) Submission (dated July 12, 2007) Regarding Fall Armyworm Resistance to the Cry1F Protein Expressed in TC1507 Herculex® I Insect Protection Maize in Puerto Rico (EPA registrations 68467-2 and 29964-3). DP Barcode: 342635. Decision: 381550. MRID#: 471760-01.

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Action Requested

BPPD[1] has been asked to review a report submitted by Dow AgroScience's (and Pioneer Hi-Bred International) that details their investigation of unexpected fall armyworm (*Spodoptera frugiperda,* FAW) damage found in TC1507 Herculex® I Insect Protection Maize fields in 2006 in Puerto Rico and whether such damage is caused by resistant insects.

[1] The use of BPPD in this review refers to BPPD IRM team consisting of Dr. Sharlene Matten, IRM Program Lead and Alan Reynolds, Entomologist.

Conclusions and Recommendations

1) Based on the results of the screening level and concentration-dependent bioassays, BPPD concludes that the unexpected performance failures of TC1507 maize observed in 2006 in Puerto Rico were due to Cry1F-resistant fall armyworm (FAW). The screening level assessment of the Cry1F-sensitivity of F1 progeny from larvae at 10,000 ng Cry1F/cm^2 revealed no significant mortality of either Puerto Rico collection on the Cry1F-treated diet. Testing of the F2 progeny did not show a significant concentration response to Cry1F nor did these progeny shown a significant reduction in growth. While the resistance ratio cannot be determined, it is estimated that the Puerto Rico populations are greater than 167-fold resistant to the Cry1F protein. This is the first documented case of field failure associated with insect resistance to a Bt crop. FAW is the most important pest of maize in Puerto Rico.

2) FAW resistance to TC1507 maize is much less likely in the continental U.S. because it can only overwinter in the extreme south of Texas and Florida, and therefore, selection in maize-growing regions exerts no long term selection pressure. However, there could be annual seasonal damage by long-distance migratory Cry1F-resistant FAW populations.

3) FAW resistance to TC1507 maize in Puerto Rico is not expected to have any impact on the durability of TC1507 maize in the continental U.S.

4) It is recommended that Dow and Pioneer implement an appropriate remedial action plan in Puerto Rico to manage FAW resistance to the Cry1F protein expressed in TC1507 maize. The submitted report discusses the framework for the proposed remedial action plan.

5) It is recommended that Dow and Pioneer cease all sales (beginning with the 2008 growing season) of Herculex ® I (TC1507) Insect Protection Maize in Puerto Rico until such time as Cry1F-susceptibility returns to baseline levels (i.e., level prior to the resistance events).

6) It is recommended that Dow and Pioneer determine whether there is fitness costs associated with FAW resistance to the Cry1F protein expressed in TC1507 maize.

7) It is recommended that Dow and Pioneer determine the genetics of the FAW resistance, the frequency of resistance (the spread of resistance), and mechanism of resistance in field populations in Puerto Rico.

8) It is recommended that Dow and Pioneer continue sampling of FAW in Puerto Rico and resistance testing each year as part of its annual resistance monitoring program until such time as FAW resistance to Cry1F has been reverted to Cry1F baseline susceptibility levels.

9) It is recommended that Dow and Pioneer provide the results of their investigations regarding FAW resistance to the Cry1F protein expressed in Herculex ® I (TC1507)

Insect Protection Maize on an annual basis beginning January 31, 2008 and annually, thereafter.

Background

TC1507 maize has been commercially available in all maize growing areas of the United States of America including the US Territory of Puerto Rico (with the exception of Maine) since 2003. Fall armyworm (FAW) is the major insect pest of maize in Puerto Rico. TC1507 maize expresses the plant-incorporated protectant, Cry1Fa protein from *Bacillus thuringiensis* var. *aizawai*, was registered by the USEPA in 2001 for use to control or suppress the following key pests: *Ostrinia nubilalis* (European corn borer, ECB), *Diatraea grandiosella* (southwestern corn borer, SWCB), and *Helicoverpa zea* (corn earworm, CEW). TC1507 maize is also labeled for use against a number of secondary pests including *Spodoptera frugiperda* (fall armyworm, FAW).

On June 21, 2007, Dow AgroSciences and Pioneer Hi-Bred met with EPA BPPD to discuss their work on *Spodoptera frugiperda* (fall armyworm) in Puerto Rico in relation to unexpected damage reports on TC1507 maize (also known as Herculex® Insect Protection Maize) reported to them in 2006 by three customers and in the Dow AgroSciences (Mycogen Seeds) research plots. Both registrants were interested in determining whether such damage was caused by resistant insects. Dow AgroSciences (and Pioneer Hi-Bred) subsequently provided EPA BPPD with a report of their investigations regarding control of FAW by TC1507 maize in Puerto Rico (dated July 12, 2007, MRID# 471760-01). This report is the subject of this technical review.

Follow-up Testing Conducted on Two Puerto Rico FAW Populations

Dow AgroSciences' personnel performed follow-up testing on the Puerto Rican populations. The following three conclusions can be reached from these investigations.

- Testing using ELISA lateral flow membrane strips confirmed that the TC1507 maize expressed the Cry1F protein.
- Testing confirmed that the unexpected damage was caused by FAW larvae feeding on TC1507 maize (company expert).
- Testing of the susceptibility to the Cry1F protein indicated that the two FAW larval populations collected from VEH Farms (a commercial farm) and Mycogen Research Farm (SI) had significantly lower susceptibility to the Cry1Fa protein than the control, Benzon colony. The screening level assessment of the Cry1a-sensitivity of F1 progeny from the field-collected larvae at 10,000 ng Cry1F/cm^2 revealed no significant mortality of either Puerto Rico collection on the Cry1F-treated diet. Testing of the F2 progeny did not show a significant concentration response to Cry1F nor did these progeny show a significant reduction in growth. The resistance ratio could not be calculated for the Puerto Rico populations because the LC_{50s} could not be determined, but it is >167. The susceptibility testing is discussed in detail below.

FAW Susceptibility Testing

In March 2007, FAW larvae were collected from two fields, one from VEH Farms and the other one from SI research plot. These collections were shipped under USDA APHIS PPQ permit (February 2007), to Dow AgriSciences' insectary in Indianapolis, IN for rearing and bioassay. During the rearing process, the VEH and SI populations were handled separately to avoid cross-contamination. From the VEH location, 164 larvae were received and 113 were reared to adulthood. From the SI location, 192 larvae were received and 163 were reared to adulthood. A control colony was established from FAW eggs from Benzon Research insectary (Carlisle, PA).

The screening level bioassay was performed using partially microbially-produced Cry1F protein (80% a.i. Lot TSN104812, Pro Cry1F, MR872 formulated in 100 mM CAPS buffer pH 11.0) at varying concentrations of 10,000, 1000, 100, and 10 ng Cry1F/cm^2 applied to the diet. Mortality was recorded after seven days. Neonate larvae from both Puerto Rico populations showed similar survival and growth when tested at the highest concentration of 10,000 ng Cry1F/cm^2 as the untreated diet. The larvae from the Benzon control colony all died at this concentration.

Third instar F1 larvae survivors from the Puerto Rico populations were transferred to individual larval rearing units containing untreated (no Cry1F) diet, allowed to develop to adulthood, and then mate. The F2 generations of the VEH and SI populations were used for concentration-response assessment. Additional FAW eggs susceptible to the Cry1F protein were obtained from Benzon Research and synchronized for larval emergence with the two Puerto Rico populations. Neonates were hatched in Petri dishes and within12 hours of hatching were then transferred to diet that was either untreated, buffer treated or surface treated with partially-purified microbially-produced Cry1F protein (see description above). A 128 well bioassay tray was used for the testing. The lowest concentration used was1.52 ng Cry1F/cm^2 and the highest concentration tested was 10,000 ng Cry1F/cm^2. Protein solutions were allowed to dry on the top of the diet at room temperature. Up to 32 wells (replicates) were treated for each concentration for each population. One neonate larva was place in each treated well using a fine brush, wells were covered with a gas permeable seal and trays were held at 25°C (16:8 L:D). At the end of the 7-day period, larvae were assessed for mortality and surviving individuals from each colony at each concentration were combined and weighed.

For the mortality endpoint, the two Puerto Rico colonies did not show a significant concentration response to Cry1F. The Benzon susceptible colony showed a concentration response, LC50 = 60 ng Cry1F/cm^2 (95% fiducial intervale = 27-205 ng ng Cry1F/cm^2) and were all killed at 3,333 ng Cry1F/ cm^2. For the growth inhibition endpoint, the Benzon colony showed a significant growth inhibition effect caused by all Cry1F concentrations tested; however, neither Puerto Rico colony showed a significant reduction in growth. No resistance ratio could be calculated for either Puerto Rico colony because the LC_{50} could not be determined. However, this ratio is greater than 167.

Remedial Action Plan

A specific remedial action plan for FAW resistance is not required under the terms and conditions of the Cry1F Bt (TC1507) maize registrations ((EPA registrations 68467-2 and 29964-3) because FAW is considered to be a secondary pest. Dow AgroSciences and Pioneer Hi-Bred have formulated a remedial action plan designed to reduce the selection pressure for FAW resistance to TC1507 maize. This plan is analogous to that required by EPA for the primary target pests of TC1507 maize: *Ostrinia nubilalis* (European corn borer), *Helicoverpa zea* (corn earworm), and *Diatraea grandiosella* (southwestern corn borer). The following steps are being taken by the registrants following confirmation of FAW resistance Puerto Rico:

1. Existing customers were notified and they are recommended to use insecticides (as needed) to control FAW damage on existing TC1507 commercial fields.
2. The two registrants are taking steps to discontinue commercial sales of TC1507 maize in Puerto Rico.
3. FAW populations will be carefully managed in maize research and production fields in Puerto Rico with insecticides.
4. Before TC1507 (Cry1F) maize is reintroduced commercially in Puerto Rico the susceptibility of FAW must be confirmed.

Storer et al. (2007) note that this remedial action plan is specific for resistant FAW to TC1507 maize in Puerto Rico and should not be seen as a precedent should such resistance occur in a labeled pest elsewhere.

Factors Contributing to TC1507 Maize Damage in Puerto Rico

Storer et al. (2007) described several unique factors that contributed to the development of Cry1F-resistant FAW in Puerto Rico. These factors include:

a) an island setting providing an isolated ecosystem that reduces insect migration;
b) a tropical climate allowing year-round maize production and therefore year-round insect pressure;
c) TC1507 maize is the Bt maize of choice because it is highly effective in controlling FAW;
d) more than 50 FAW generations have been continually exposed to Cry1F Bt maize (TC1507 maize) since its introduction into Puerto Rico in 2003; and
e) a high population and drought conditions in 2006 funneled a large percentage of insects through irrigated TC1507 maize (FAW larvae migrate from declining quality host plants).

Potential for FAW Resistance in the Continental U.S.

Storer et al. (2007) describe the limiting factors that reduce the likelihood of FAW resistance in the continental U.S. FAW is a migratory seasonal pest. The species cannot develop at temperatures below 50°F and as a tropical species, do not diapause. FAW only survive winter in the extreme south of Texas and Florida and thus, selection for Cry1F-resistance in maize-

growing areas exerts no long-term selection pressure. In the continental U.S., there are only one or two generations per year on maize. Alternate host crops, which include primarily grasses, are abundant. FAW is not a key target pest of Bt maize (SRM note: except for Bt sweet corn). Both Cry1F- and Cry1Ab-expressing maize are planted in the continental U.S. and this market mix reduces the selection pressure on either protein. Storer et al. (2007) provide a published study (Luo et al. 1999) that shows that there is a limited potential for cross-resistance. Finally, there have been no reports of FAW performance problems on TC1507 maize in the continental U.S.

FAW Situation in Puerto Rico Does Not Parallel the Situations for Key Target Pests on the Mainland

TC1507 produces a high dose of Cry1F against European corn borer and southwestern corn borer. It would be expected that any partially-resistant (heterozygotes) ECB or SWCB would not be able to survive on TC1507 plants and pass their genes for resistance to the next generation. For CEW, the large number of alternate crop and non-crop hosts provides natural unstructured refuge, in addition, to the structured refuge. None of these pests possess the larval migratory behavior of FAW; this reduces the chance that heterozygotes or partially-resistant individuals could obtain a fitness advantage over fully susceptible individuals. Simulation modeling for ECB, SWCB, and CEW resistance to Bt maize predicts that a 20% structured refuge will provide long-term durability of TC1507 maize. Market competition, i.e., Cry1Ab- and Cry1F-expressing Bt maize products, serve to dilute the selection pressure for Cry1F resistance.

BPPD Review

The screening level assessment of the Cry1F-sensitivity of F1 progeny from the field-collected larvae at 10,000 ng Cry1F/cm^2 revealed no significant mortality of either Puerto Rico collection (VEH and SI populations) on the Cry1F-treated diet. Third instar F1 larvae survivors from the Puerto Rico populations were transferred to individual larval rearing units containing untreated (no Cry1F) diet, allowed to develop to adulthood, and then mate. The F2 generations of the VEH and SI populations were used for concentration-response assessment. Testing of the F2 progeny did not show a significant concentration response to Cry1F nor did these progeny show a significant reduction in growth. The resistance ratio could not be calculated for the Puerto Rico populations, but it is greater than 167 [$= \frac{\geq 10{,}000 \text{ ng Cry1F/cm}^2}{60 \text{ ng Cry1F/cm}^2}$].

Based on the results of the screening level bioassay and concentration-dependent bioassay, BPPD concludes that the unexpected performance failures observed in 2006 in Puerto Rico were due to Cry1F-resistant FAW feeding on TC1507 maize. This is the first documented case of field failure associated with insect resistance to a Bt crop. FAW is the most important pest of maize in Puerto Rico. Herculex® I (TC1507) Insect Protection Maize cannot be used effectively to control FAW in Puerto Rico. It is recommended that all sales of TC1507 maize be ceased in Puerto Rico until Cry1F-susceptibility returns to baseline levels (i.e., level prior to the resistance events).

BPPD agrees with Storer et al. (2007) that there were many important factors that increased the selection pressure for FAW resistance to TC1507 maize in Puerto Rico. It is agreed that the proposed remedial action plan is specific for Cry1F-resistant FAW populations in Puerto Rico and is not a precedent should Cry1F-resistance occur in the continental U.S. The Cry1F-resistance potential for ECB, SWCB, and CEW is quite different than that of FAW. Simulation modeling for ECB, SWCB, and CEW resistance to Bt maize predicts that a 20% structured refuge will provide long-term durability of TC1507 maize.

The circumstances that led to FAW resistance in Puerto Rico do not exist in the continental U.S. FAW is a sporadic, secondary pest that can only survive the winter in the extreme south of Texas and Florida and, therefore, there is no long-term selection pressure in the maize growing regions. The market mix of both Cry1b- and Cry1F-expressing Bt maize products reduces the selection pressure on either protein. Binding studies by Luo et al. (1999) using brush border membrane membranes from FAW and *Spodoptera exigua* suggests that there are at least two high affinity binding sites, one site is for Cry1Ac and the other is for Cry1Bb and Cry1Ca. Furthermore, Luo et al. showed that Cry1Fa competes for both the Cry1Ac binding site and the Cry1Bb and Cry1Ca site. This research suggests that while there is cross-resistance potential, it is limited. Finally, there have been no reports of FAW performance problems on TC1507 maize in the continental U.S. FAW resistance to TC1507 maize in Puerto Rico, therefore, is not expected to have a significant impact on the durability of TC1507 maize in the continental U.S.

While there is a potential for significant fitness costs to be associated with resistance that limit the ability of resistant insects to propagate once the selection pressure is removed; we do not know if this is the case for the Cry1F-resistant FAW populations in Puerto Rico. We also do not know anything about the genetics of resistance, the resistance allele frequency, the spread of resistance, or the mechanism of resistance. This information is critical to understanding FAW resistance to the Cry1F protein expressed in TC1507 maize in Puerto Rico and whether it is possible for a return to susceptibility of FAW.

See the **Conclusions and Recommendations** section for specific recommendations.

References

Luo, K., D. Banks, and M.J. Adang. 1999. Toxicity, binding, and permeability analyses of four *Bacillus thuringiensis* Cry1 δ-endotoxins using brush border membrane vesicles of *Spodoptera exigua* and *Spodoptera frugiperda*. Appl. Env. Microbiol. 65: 457-464.

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Chemical:	**Bacillus thuringiensis Cry 1F protein and the genetic material necessary for its production (plasmid insert PHI 8999)in corn** **Bacillus thuringiensis moCry 1F insecticidal protein and the genetic material necessary for its production in maize**
	PC Code: **006481** **006491**
HED File Code:	**41400 BPPD IRM**
Memo Date:	**8/24/2007**
File ID:	**DPD342635**
Accession #:	**000-00-9003**

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