Bacillus thuringiensis Cry1Ab Delta-Endotoxin Protein and the Genetic Material Necessary for Its Production (via Elements of Vector pZO1502) in Event *Bt*11 Corn (OECD Unique Identifier: SYN-BTØ11-1)(006444) & *Bacillus thuringiensis* Vip3Aa20 Insecticidal Protein and the Genetic Material Necessary for Its Production (*via* Elements of Vector pNOV1300) in Event MIR162 Maize (OECD Unique Identifier: SYN-IR162-4)(006599) & Modified Cry3A Protein and the Genetic Material Necessary for Its Production (via Elements of Vector pZM26) in Event MIR604 Corn (OECD Unique Identifier: SYN-IR6Ø4-5)(006509) Fact Sheet

Summary

The Environmental Protection Agency (EPA) has conditionally registered a plant-incorporated protectant (PIP) product containing Syngenta Seeds, Incorporated's (hereafter referred to as Syngenta) new active ingredient, *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (*via* elements of vector pNOV1300) in Event MIR162 maize (Organization for Economic Cooperation and Development [OECD] Unique Identifier: SYN-IR162-4). This new product, *Bt*11 x MIR162 x MIR604 corn (expressing previously registered Cry1Ab, Vip3Aa20, previously registered mCry3A, respectively), is intended for commercial distribution and use. The Agency has determined that the use of this pesticide is in the public interest and that it will not cause any unreasonable adverse effects on the environment during the time of conditional registration. The registrant for this product is Syngenta.

Bt 11 x MIR162 x MIR604 corn produces its own insecticidal proteins within the corn plant. These insecticidal proteins were derived from *Bacillus thuringiensis* (*Bt*), a naturally occurring soil bacterium. The Cry1Ab and the Vip3Aa20 insecticidal proteins expressed in this product control certain lepidopteran pests of corn, while the mCry3A insecticidal protein controls coleopteran pests of corn.

On August 6, 2008, a tolerance exemption under 40 Code of Federal Regulations (CFR) Part 174 became effective for Vip3Aa proteins, when used as plant-incorporated protectants, in or on corn and

cotton (40 CFR § 174.501). The exemption from the requirement of a tolerance for residues of Vip3Aa proteins is inclusive of the Vip3Aa20 insecticidal protein and its use in corn.

I. Target Pests/ Application Sites & Methods

- **Pesticide Name:** *Bacillus thuringiensis* Cry1Ab delta-endotoxin protein and the genetic material necessary for its production (*via* elements of vector pZO1502) in Event *Bt*11 corn (OECD Unique Identifier: SYN-BTØ11-1) x *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (*via* elements of vector pNOV1300) in Event MIR162 maize (OECD Unique Identifier: SYN-IR162-4) x modified Cry3A protein and the genetic material necessary for its production (*via* elements of vector pZM26) in Event MIR604 corn (OECD Unique Identifier: SYN-IR6Ø4-5)

- **Date Registered:** February 13, 2009

- **Registration Numbers:** 67979-13

- **Trade and Other Names:** *Bt*11 x MIR162 x MIR604 Corn or Agrisure™ 3100

- **PC Codes:** 006444(Cry1Ab), 006599 (Vip3Aa20), and 006509 (mCry3A)

- **Basic Manufacturers:**

  Syngenta Seeds, Incorporated – Field Crops – NAFTA
  P.O. Box 12257, 3054 East Cornwallis Road
  Research Triangle Park, NC 27709-2257

- **Type of Pesticide:** Plant-Incorporated Protectant

- **Uses:** Field Corn

- **Target Pest(s):** European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), southern cornstalk borer (*Diatraea crambidoides*), corn earworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), armyworm (*Pseudaletia unipunctata*), beet armyworm (*Spodoptera exigua*), black cutworm (*Agrotis ipsilon*), western bean cutworm (*Striacosta albicosta*), sugarcane borer

(*Diatraea saccharalis*), common stalk borer (*Papaipema nebris*), western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*Diabrotica barberi*), and Mexican corn rootworm (*Diabrotica virgifera zeae*)

II. Science Assessment

A. Product Characterization

Vip3A is a group of vegetative insecticidal proteins (i.e., produced during the vegetative stage of bacterial growth) from *Bacillus thuringiensis*, a gram-positive bacterium commonly found in soil. Event MIR162 maize, produced by *Agrobacterium*-mediated transformation using elements of a vector (pNOV1300), contains a variant of the native *vip3Aa1* gene, which was isolated from *Bt* strain AB88. The gene encodes a vegetative insecticidal protein, Vip3Aa20, that is highly toxic to the following lepidopteran pests of corn: fall armyworm (*Spodoptera frugiperda*), armyworm (*Pseudaletia unipunctata*), beet armyworm (*Spodoptera exigua*), corn earworm (*Helicoverpa zea*), black cutworm (*Agrotis ipsilon*), and western bean cutworm (*Striacosta albicosta)*. Event MIR162 maize also contains the *manA* gene from *Escherichia coli*, which encodes the selectable marker, phosphomannose isomerase (PMI).

Southern blot analysis was used to confirm the presence of the *cry1Ab* and *pat* genes from the parental Event *Bt*11, *vip3Aa20* and *pmi* genes from the parental Event MIR162, and *mcry3A* and *pmi* genes from the parental Event MIR604 in *Bt*11 x MIR162 x MIR604 corn. Samples from *Bt*11 x MIR162 x MIR604 corn gave the same results as those observed for the individual parental events, indicating that the molecular characterization data provided for the individual parental events are also applicable to *Bt*11 x MIR162 x MIR604 corn.

Protein expression data, together with data indicating that the insecticidal proteins in Events *Bt*11, MIR162, and MIR604 act individually to effect a typical midgut pathology in susceptible insects like previously studied *Bt* delta-endotoxins, demonstrate that no synergistic action or interaction of these insecticidal proteins is known or expected to occur. Thus, the data on the individual events and individual insecticidal proteins can be used to support the safety of *Bt*11 x MIR162 x MIR604 corn.

B. Human Health Assessment

There is a reasonable certainty that no harm will result from aggregate exposure to the United States (U.S.) population, including infants and children, to the Vip3Aa20

insecticidal protein. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because no toxicity to mammals has been observed, nor any indication of allergenicity potential for the plant-incorporated protectant.

Syngenta previously submitted four acute oral toxicity studies conducted on mice, which all indicated that Vip3Aa proteins are non-toxic to humans. Three of the studies were conducted with microbially produced Vip3Aa proteins with slight variations in amino acid sequence (1–2 amino acid differences), and one study was conducted with protein extracted from transgenic corn leaf tissue as the test material. No treatment-related adverse effects were observed in any of the studies. The oral LD_{50} for mice (males, females, and combined) was greater than 3,675 milligrams (mg) Vip3Aa/kilogram (kg) body weight (the highest dose tested). Additionally, Syngenta submitted another mouse acute oral toxicity study that showed no effects attributed to Vip3Aa20 insecticidal protein, even at relatively high dose levels (1,250 mg Vip3Aa20/kg body weight).

Since Vip3Aa isolates are proteins, allergenic potential was also considered. Currently, no definitive tests for determining the allergenic potential of novel proteins exist. Therefore, EPA uses a weight-of-evidence approach where the following factors are considered: source of the trait; amino acid sequence comparison with known allergens; and biochemical properties of the protein, including *in vitro* digestibility in simulated gastric fluid (SGF) and glycosylation. This approach is consistent with the approach outlined in the Annex to the Codex Alimentarius "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants." The allergenicity assessment for Vip3Aa proteins is as follows:

1. Source of the trait. *Bacillus thuringiensis* is not considered to be a source of allergenic proteins.

2. Amino acid sequence. A comparison of the amino acid sequence of Vip3Aa20 with known allergens showed no significant sequence identity over 80 amino acids or identity at the level of eight contiguous amino acid residues.

3. Digestibility. The Vip3Aa proteins were digested rapidly in simulated gastric fluid containing pepsin.

4. Glycosylation. Vip3Aa proteins were shown not to be glycosylated.

5. Conclusion. Considering all of the available information, EPA has concluded that the potential for Vip3Aa proteins to be food allergens is minimal.

With respect to the previously registered plant-incorporated protectants that have been combined with Vip3Aa20 to create *Bt*11 x MIR162 x MIR604 corn, the following web links provide access to Biopesticides Registration Action Documents that contain comprehensive human health assessments for each active ingredient:

C. Environmental Assessment

Presently, the Agency is aware of no identified significant adverse effects of Vip3Aa proteins on the abundance of non-target beneficial organisms in any population in the field environment, whether they are pest parasites, pest predators, or pollinators. Further, the EPA believes cultivation of Event MIR162 maize may have fewer adverse impacts on non-target organisms than the use of chemical pesticides for maize production, because under normal circumstances, MIR162 maize requires substantially fewer applications of chemical pesticides, compared to production of non-*Bt* maize. Fewer chemical insecticide applications generally result in increased populations of beneficial organisms that control secondary pests, such as aphids and leafhoppers. In addition, no adverse effect on Federally listed endangered and threatened species is expected from the proposed lepidopteran-resistant maize registration. Furthermore, the EPA has determined that there is no significant risk of gene capture and expression of Vip3Aa proteins by wild or weedy relatives of corn in the U.S., its possessions, or its territories. Available data do not indicate that Vip3Aa proteins have any measurable adverse effect on microbial populations in the soil, nor has any horizontal transfer of genes from transgenic plants to soil bacteria been demonstrated. In conclusion, the risk assessment finds no hazard to the environment from cultivation of Event MIR162 maize expressing Vip3Aa insecticidal protein.

Prior environmental assessments for the Cry1Ab and mCry3A insecticidal proteins can be found at the web links provided in the "Human Health Assessment" section above.

The synergism studies, non-target organism toxicity testing, and field studies reviewed for the *Bt*11, MIR162, and MIR604 parental events indicate their associated combined-PIP product, *Bt*11 x MIR162 x MIR604 corn, will not result in any unexpected interaction related to an antagonistic or synergistic action to target and non-target insects. Therefore, it is extremely unlikely that the Cry1Ab, Vip3Aa,

and mCry3A insecticidal proteins contained in a single plant will impart any hazard to non-target organisms exposed to the *Bt*11 x MIR162 x MIR604 corn hybrid in the environment.

Furthermore, the compilation of ecotoxicity studies on non-target organisms, evaluation for synergism between the test proteins, efficacy data, and field data support the bridging of the environmental risk assessment from the parental events to the combined-PIP product. Based on prior assessments conducted on the Cry1Ab, Vip3Aa, and mCry3A insecticidal proteins individually, the environmental risk assessment for the *Bt*11 x MIR162 x MIR604 corn hybrid indicates that no unreasonable harm will result to the environment or any Federally listed endangered or threatened species from commercial cultivation of this corn hybrid.

D. Insect Resistance Management

*Bt*11 x MIR162 x MIR604 corn requires an insect resistance management program because it is intended for unlimited commercial distribution. In order to reduce the possibility of the target pests developing resistance to the Cry1Ab, Vip3Aa20, and mCry3A insecticidal proteins expressed in *Bt*11 x MIR162 x MIR604 corn, EPA is requiring Syngenta to ensure that a portion of the planted acreage of this product be set aside where non-*Bt* corn, non-lepidopteran-resistant *Bt* corn, and/or non-corn rootworm-protected *Bt* corn (depending on the refuge option employed) will be grown to serve as a "refuge." The refuge requirements for *Bt*11 x MIR162 x MIR604 corn are described below.

Two options for deployment of the refuge are available to growers.

The first option is planting a common refuge for both corn borers and corn rootworms. The common refuge must be planted with corn hybrids that do not contain *Bt* technologies for the control of corn rootworms or corn borers. The refuge area must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and refuge acres). It must be planted as a block adjacent to the *Bt*11 x MIR162 x MIR604 corn field, perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. If the common refuge is planted on rotated ground, then *Bt*11 x MIR162 x MIR604 corn must also be planted on rotated ground. If the common refuge is planted in continuous corn, the *Bt*11 x MIR162 x MIR604 corn field may be planted on either continuous or rotated land. The common refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae and other soil pests. The refuge can also be treated with a non-*Bt*

foliar insecticide for control of late season pests, if pest pressure reaches an economic threshold for damage; however, if rootworm adults are present at the time of foliar applications, then the *Bt*11 x MIR162 x MIR604 corn field must be treated in a similar manner. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants). Pests other than adult corn rootworms can be treated with an appropriate pest-labeled insecticide on the common refuge acres without treating the *Bt*11 x MIR162 x MIR604 corn acres only if treatment occurs when adult corn rootworms are not present. Pests on the *Bt*11 x MIR162 x MIR604 corn acres can be treated as needed without having to treat the common refuge.

The second option is planting separate refuge areas for corn borers and corn rootworms. The corn borer refuge must be planted with a non-*Bt*/lepidopteran-protected hybrid, must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and corn borer refuge acres), and must be planted within ½ mile of the *Bt*11 x MIR162 x MIR604 corn field. Refuge planting options include separate fields, blocks within fields (e.g., along the edges or headlands), perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. The corn borer refuge can be treated with a soil-applied or seed-applied insecticide for corn rootworm larval control or a non-*Bt* foliar-applied insecticide for corn borer control, if pest pressure reaches an economic threshold for damage. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants).

The corn rootworm refuge must be planted with a non-*Bt*/corn rootworm-protected hybrid, but can be planted with *Bt* corn hybrids that control corn borers. The corn rootworm refuge must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and corn rootworm refuge acres) and must be planted as an adjacent block, perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. If the rootworm refuge is planted on rotated ground, then *Bt*11 x MIR162 x MIR604 corn must also be planted on rotated ground. If the rootworm refuge is planted in continuous corn, the *Bt*11 x MIR162 x MIR604 corn field may be planted on either continuous or rotated land. More generally, the rootworm refuge should utilize comparable agronomic practices as the *Bt*11 x MIR162 x MIR604 corn acres. The corn rootworm refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae

and other soil pests. The refuge can also be treated with a non-*Bt* foliar insecticide for control of late season pests; however, if rootworm adults are present at the time of foliar applications, then the *Bt*11 x MIR162 x MIR604 corn field must be treated in a similar manner. Pests other than adult corn rootworms can be treated on the rootworm refuge acres without treating the *Bt*11 x MIR162 x MIR604 corn acres only if treatment occurs when adult corn rootworms are not present or if a pesticide without activity against adult corn rootworms is used. Pests on the *Bt*11 x MIR162 x MIR604 corn acres can be treated as needed without having to treat the rootworm refuge.

BPPD has concluded that based on the modeling, dose, and efficacy studies, the refuge requirements, as described above, are acceptable for *Bt*11 x MIR162 x MIR604 corn. Syngenta will also be required to conduct a resistance monitoring program for Cry1Ab, Vip3Aa20, and mCry3A with the major target pests. Additional requirements for remedial action (in the event of resistance), grower agreements, grower education, compliance assurance, and annual reports have also been implemented for *Bt*11 x MIR162 x MIR604 corn as terms of registration.

E. Benefits

Field and efficacy trials have demonstrated that MIR162 maize, expressing Vip3Aa20 insecticidal protein, effectively controls a wide spectrum of lepidopteran pests: fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), western bean cutworm (*Striacosta albicosta*), and black cutworm (*Agrotis ipsilon*). The field trials showed that the level of protection provided by MIR162 maize against the aforementioned pests is significantly better than that provided by currently registered *Bt*11 corn alone or a negative isoline with a conventional insecticide standard. However, MIR162 maize is not intended for commercial distribution (i.e., individual-trait seed is not to be used for grain production or for protection from lepidopteran pests) but for use in creating combinations with other registered PIPs, such as the *Bt*11 and MIR604 traits, that will be marketed to participants in the agricultural industry. For example, pyramided and stacked *Bt*11 x MIR162 x MIR604 corn, which showed reasonably good efficacy against western corn rootworm, European corn borer and the above-mentioned lepidopteran pests, would provide a new tool for farmers who face damage pressures from lepidopteran and coleopteran pests. Additionally, the Vip3Aa20 insecticidal protein has not been previously registered and provides a unique mode of action, expresses a high dose against fall armyworm and a "near high dose" against corn earworm, and has a low likelihood of cross-resistance with other *Bt* Cry proteins. All of these unique characteristics may benefit insect resistance management for this and other corn PIP products.

Furthermore, as another registered *Bt* corn product, *Bt*11 x MIR162 x MIR604 corn will likely result in direct and indirect human and environmental health benefits by providing growers with an alternative *Bt* corn option and the potential to increase grower choice and price competition, resulting in lower seed prices for growers and higher adoption rates. Registration of *Bt*11 x MIR162 x MIR604 corn may also result in further reduction of chemical insecticide use by growers.

F. Public Interest Finding

To grant a conditional registration under Section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), EPA must determine that such conditional registration will, *inter alia*, be in the public interest. EPA determines whether conditional registration of a pesticide is in the public interest in accordance with the criteria set forth at 51 Federal Register (FR) 7628 (*Conditional Registration of New Pesticides*; March 5, 1986). Based on analysis that applies these criteria, EPA concludes that the use of *Bt*11 x MIR162 x MIR604 corn will be in the public interest. Utilization of this product will result in direct and indirect human and environmental health benefits by providing growers with an additional *Bt* corn product and the potential to extend the useful life of *Bt* corn technology, generally due to a novel mode of action (Vip3Aa20) and low likelihood of cross-resistance with other *Bt* Cry proteins.

Terms and Conditions of the Registration

The following terms and/or conditions are required for the FIFRA section 3(c)(7)(C) registration of *Bt*11 x MIR162 x MIR604 corn:

0. The subject registration will automatically expire at midnight on December 31, 2011.

1. The subject registration will be limited to Cry1Ab (*Bacillus thuringiensis* Cry1Ab delta- endotoxin protein and the genetic material necessary for its production [via elements of vector pZO1502] in Event *Bt*11 corn [OECD Unique Identifier: SYN-BTØ11-1]) x Vip3Aa20 (*Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production [via elements of vector pNOV1300] in Event MIR162 maize [OECD Unique Identifier: SYN-IR162-4]) x mCry3A (modified Cry3A protein and the genetic material necessary for its production [via elements of vector pZM26] in Event MIR604 corn [OECD Unique Identifier: SYN-IR6Ø4-5]) for use in field corn.

2. Syngenta will submit/cite all data required for registration of their product under FIFRA section 3(c)(5) when the Agency requires all registrants of similar products to submit such data.

3. Syngenta will submit/cite all data required to support the individual plant-incorporated protectants in YieldGard® Insect Resistant Corn, MIR162 maize, and Agrisure® RW Rootworm-Protected Corn within the time frames required by the terms and conditions of EPA Registration Numbers 67979-1, 67979-14, and 67979-5, respectively:

Study Type [**] **Registration**	**Required Data**	**Due Date**
Residue Analytical Method – Plants (Harmonized Test Guideline 860.1340) [**]MIR162 maize	The validation of the analytical method performed by Syngenta (as described in Standard Operating Procedure 2.91) must provide the following: (1) results as a concentration (i.e., gram/gram) as opposed to an optical density value and (2) testing on dilutions from corn samples, before grinding, instead of flour samples in order to address variability introduced by grinding and sample preparation. Additionally, Syngenta must provide to the EPA laboratory (Ft. Meade, Maryland) methodology and/or reagents necessary for validation of such analytical method within six months from the date that the Agency requests them.	November 1, 2009
Aquatic Invertebrate Toxicity (Harmonized Test Guideline 885.4240) [**]MIR162 maize	A 7–14 day *Daphnia* study as per the Harmonized Test Guideline 885.4240 guideline must be submitted as a condition of registration. Alternatively, a dietary study of the effects on an aquatic invertebrate, representing the functional group of a leaf shredder in headwater streams, can be performed and submitted in lieu of the 7–14 day *Daphnia* study.	November 1, 2009
Insect Resistance Management – Annual Reporting [**]MIR162 maize	Annual sales data, to include units sold and acres planted, must be reported and summed by state and county.	January 31st of each year, beginning in 2010
Simulated or Actual Field Tests – Non-Target Invertebrates [**]Agrisure® RW Rootworm-Protected Corn	Three (3) year full-scale field or semi-field studies for evaluation of mCry3A Event MIR604 corn exposure on non-target invertebrates must be conducted and a final report submitted. Full-scale field experiments must be appropriately designed to provide a measure of ecological impacts (larger fields, more replicates, more samples per plot based on recommendations of the August 2002 Scientific Advisory Panel [SAP] and the subsequent relevant research on appropriate study design).	January 31, 2011
Field Degradation Studies [**]Agrisure® RW Rootworm-Protected Corn	Field degradation studies evaluating accumulation and persistence of mCry3A in several soils and various strata must be conducted and a final report, regarding data from fields that have had three continuous years of cultivation of Event MIR604 corn, submitted. Representative fields must have been planted with mCry3A corn, include both conventional tillage and no-till samples, and be harvested under typical agronomic conditions. Sampling must continue until the limit of detection is reached. Studies should include soils with high levels of a variety of clays. Both enzyme-linked immunosorbent assay (ELISA) and insect bioassays need to be conducted to determine if mCry3A is accumulating or persisting in soil	January 31, 2011

samples.

4. Syngenta must submit the following data and/or information in the time frames listed:

Study Type	Required Data	Due Date
Insect Resistance Management – Dose	Because of the potential for synergistic interactions between plant-incorporated protectants in a stacked product, field efficacy studies and/or a protein expression report for SWCB, which show that *Bt*11 x MIR162 x MIR604 corn has the same dose profile as its single trait products, must be submitted as confirmatory data.	March 1, 2010
Insect Resistance Management – Grower Agreement	A copy of the grower agreement, associated stewardship documents, and written description of a system, which assures that growers will sign grower agreements and persons purchasing *Bt*11 x MIR162 x MIR604 corn will annually affirm that they are contractually bound to comply with the requirements of the IRM program, must be submitted.	Within 90 days of the date of registration
Insect Resistance Management – Compliance Monitoring Program	A CAP for *Bt* 11 x MIR162 x MIR604 corn must be submitted and must include a "phased compliance approach" that outlines instances of non-compliance to the IRM requirements and options of responding to non-compliant growers. This compliance assurance program should be harmonized with compliance assurance programs already in place for previously registered Syngenta *Bt* corn products.	Within 90 days of the date of registration
Insect Resistance Management – Resistance Monitoring	Baseline susceptibility and diagnostic concentration determinations for SWCB and CEW to Vip3Aa20 must be submitted.	August 31, 2010
Insect Resistance Management – Resistance Monitoring	A detailed Vip3Aa20 resistance monitoring plan, integrating standard procedures developed by the ABSTC and similar in structure to those established for previously registered Syngenta *Bt* corn products, for the key target pests of CEW and SWCB must be submitted.	Within 90 days of the date of registration
Insect Resistance Management – Resistance Monitoring	A revised mCry3A resistance monitoring program that incorporates *Bt*11 x MIR162 x MIR604 corn must be submitted. <u>Consideration for corn rootworm (CRW):</u> In addition to mortality assays, consider utilizing sublethal bioassays (e.g., head capsule measurements) and molecular marker methods for CRW monitoring.	Within 90 days of the date of registration
Insect Resistance Management – Resistance Monitoring	Submit data generated by the following actions: (a) initiate establishment of CRW strains that are resistant to mCry3A and investigate the nature, inheritance, and fitness costs of specific mechanisms of resistance to mCry3A, (b) study the behavioral deterrence (avoidance) mechanism further, and (c) continue studies on the biological impact of CRW adults surviving on corn expressing the mCry3A toxin.	January 31, 2010
Insect Resistance Management – Resistance Monitoring	Develop, validate, and submit an appropriate discriminating or diagnostic dose assay for the mCry3A resistance monitoring program.	January 31, 2010

Insect Resistance Management – Resistance Monitoring	Finalize and submit rootworm damage guidelines for the mCry3A resistance monitoring program.	January 31, 2010
Insect Resistance Management – Remedial Action Plan	A final remedial action plan for the Vip3Aa20 toxin expressed in *Bt*11 x MIR162 x MIR604 corn, integrating the standard procedures developed by the ABSTC and harmonized with remedial action plans established for previously registered Syngenta *Bt* corn products, must be submitted.	Within 90 days of the date of registration

5. The insect resistance management terms and conditions for *Bt*11 x MIR162 x MIR604 corn are as follows.

The required IRM program for *Bt*11 x MIR162 x MIR604 corn must have the following elements:

- Requirements relating to creation of a non-Bt corn and/or non-lepidopteran-resistant Bt corn refuge in conjunction with the planting of any acreage of Bt11 x MIR162 x MIR604 corn;

- Requirements for Syngenta to prepare and require Bt11 x MIR162 x MIR604 corn users to sign "grower agreements," which impose binding contractual obligations on the grower to comply with the refuge requirements;

- Requirements regarding programs to educate growers about IRM requirements;

- Requirements regarding programs to evaluate and promote growers' compliance with IRM requirements;

- Requirements regarding programs to evaluate whether there are statistically significant and biologically relevant changes in target insect susceptibility to Vip3Aa20, Cry1Ab, and/or mCry3A proteins in the target insects;

- Requirements regarding a "remedial action plan," which contains measures Syngenta would take in the event that any field-relevant insect resistance was detected as well as to report on activity under the plan to EPA;

- Annual reports on units sold by state (units sold by county level will be made available to the Agency upon request), IRM grower agreements

results, and the compliance assurance program including the educational program on or before January 31st each year, beginning in 2010.

h. **Refuge Requirements for *Bt*11 x MIR162 x MIR604 Field Corn**

These refuge requirements do not apply to seed increase/propagation of inbred and hybrid seed corn up to a total of 20,000 acres per county and up to a combined U.S. total of 250,000 acres per PIP active ingredient per registrant per year.

Grower agreements (also known as stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.

Two options for deployment of the refuge are available to growers.

The first option is planting a common refuge for both corn borers and corn rootworms. The common refuge must be planted with corn hybrids that do not contain *Bt* technologies for the control of corn rootworms or corn borers. The refuge area must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and refuge acres). It must be planted as a block adjacent to the *Bt*11 x MIR162 x MIR604 corn field, perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. If the common refuge is planted on rotated ground, then *Bt*11 x MIR162 x MIR604 corn must also be planted on rotated ground. If the common refuge is planted in continuous corn, the *Bt*11 x MIR162 x MIR604 corn field may be planted on either continuous or rotated land. The common refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae and other soil pests. The refuge can also be treated with a non-*Bt* foliar insecticide for control of late season pests, if pest pressure reaches an economic threshold for damage; however, if rootworm adults are present at the time of foliar applications, then the *Bt*11 x MIR162 x MIR604 corn field must be treated in a similar manner. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants). Pests other than adult corn rootworms can be treated with an appropriate pest-labeled insecticide on the common refuge acres without treating the *Bt*11 x MIR162 x MIR604 corn acres only if treatment occurs when adult corn rootworms are not

present. Pests on the *Bt*11 x MIR162 x MIR604 corn acres can be treated as needed without having to treat the common refuge.

The second option is planting separate refuge areas for corn borers and corn rootworms. The corn borer refuge must be planted with a non-*Bt*/lepidopteran-protected hybrid, must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and corn borer refuge acres), and must be planted within ½ mile of the *Bt*11 x MIR162 x MIR604 corn field. Refuge planting options include separate fields, blocks within fields (e.g., along the edges or headlands), perimeter strips, or in-field strips. If perimeter or in- field strips are implemented, the strips must be at least four consecutive rows wide. The corn borer refuge can be treated with a soil-applied or seed-applied insecticide for corn rootworm larval control or a non-*Bt* foliar-applied insecticide for corn borer control, if pest pressure reaches an economic threshold for damage. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants).

The corn rootworm refuge must be planted with a non-*Bt*/corn rootworm-protected hybrid, but can be planted with *Bt* corn hybrids that control corn borers. The corn rootworm refuge must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and corn rootworm refuge acres) and must be planted as an adjacent block, perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. If the rootworm refuge is planted on rotated ground, then *Bt*11 x MIR162 x MIR604 corn must also be planted on rotated ground. If the rootworm refuge is planted in continuous corn, the *Bt*11 x MIR162 x MIR604 corn field may be planted on either continuous or rotated land. More generally, the rootworm refuge should utilize comparable agronomic practices as the *Bt*11 x MIR162 x MIR604 corn acres. The corn rootworm refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae and other soil pests. The refuge can also be treated with a non-*Bt* foliar insecticide for control of late season pests; however, if rootworm adults are present at the time of foliar applications, then the *Bt*11 x MIR162 x MIR604 corn field must be treated in a similar manner. Pests other than adult corn rootworms can be treated on the rootworm refuge acres without treating the *Bt*11 x MIR162 x MIR604 corn acres only if treatment occurs when adult corn rootworms are not present or if a pesticide without activity against adult corn rootworms is used. Pests on the *Bt*11 x MIR162 x MIR604 corn

acres can be treated as needed without having to treat the rootworm refuge.

i. **Grower Agreement for *Bt*11 x MIR162 x MIR604 Corn**

1. Persons purchasing *Bt*11 x MIR162 x MIR604 corn must sign a grower agreement. The term "grower agreement" refers to any grower purchase contract, license agreement, or similar legal document.

2. The grower agreement and/or specific stewardship documents referenced in the grower agreement must clearly set forth the terms of the current IRM program. By signing the grower agreement, a grower must be contractually bound to comply with the requirements of the IRM program.

3. Syngenta must implement a system (equivalent to what is already approved for previously registered Syngenta *Bt* corn products), which is reasonably likely to assure that persons purchasing *Bt*11 x MIR162 x MIR604 corn will affirm annually that they are contractually bound to comply with the requirements of the IRM program. A description of the system must be submitted to EPA within 90 days from the date of registration.

4. Syngenta must use an approved grower agreement and must submit to EPA, within 90 days from the date of registration, a copy of that agreement and any specific stewardship documents referenced in the grower agreement. If Syngenta wishes to change any part of the grower agreement or any specific stewardship documents referenced in the grower agreement that would affect either the content of the IRM program or the legal enforceability of the provisions of the agreement relating to the IRM program, thirty days prior to implementing a proposed change, Syngenta must submit to EPA the text of such changes to ensure that it is consistent with the terms and conditions of this registration.

5. Syngenta must implement an approved system (equivalent to what is already approved for previously registered Syngenta *Bt* corn products), which is reasonably likely to assure that persons purchasing *Bt*11 x MIR162 x MIR604 corn sign grower agreement(s). A description of the system must be submitted to EPA within 90 days from the date of registration.

6. Syngenta shall maintain records of all *Bt*11 x MIR162 x MIR604 corn grower agreements for a period of three years from December 31st of the year in which the agreement was signed.

7. Beginning on January 31, 2010 and annually thereafter, Syngenta shall provide EPA with a report on the number of units of *Bt*11 x MIR162 x MIR604 corn seed shipped and not returned, and the number of such units that were sold to persons who have signed grower agreements. The report shall cover the time frame of a twelve-month period. Note: The first report shall contain the specified information from the time frame starting with the date of registration and extending through the 2009 growing season.

8. Syngenta must allow a review of the grower agreements and grower agreement records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including names, personal information, and grower license number, will be protected.

j. **IRM Education and IRM Compliance Monitoring Program for *Bt*11 x MIR162 x MIR604 Corn**

0. Syngenta must design and implement a comprehensive, ongoing IRM education program designed to convey to *Bt*11 x MIR162 x MIR604 corn users the importance of complying with the IRM program. The education program shall involve the use of multiple media, e.g. face-to-face meetings, mailing written materials, EPA-reviewed language on IRM requirements on the bag or bag tag, and electronic communications such as by internet, radio, or television commercials. Copies of the materials will be provided to EPA for their records. The program shall involve at least one written communication annually to each *Bt*11 x MIR162 x MIR604 corn user separate from the grower technical guide. The communication shall inform the user of the current IRM requirements. Syngenta shall coordinate its education program with the educational efforts of other registrants and other organizations, such as the National Corn Growers Association and state extension programs.

1. Annually, Syngenta shall revise, and expand as necessary, its education program to take into account the information collected through the compliance survey required under paragraph 6 and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high.

2. Beginning January 31, 2010, Syngenta must provide a report to EPA summarizing the activities it carried out under its education program for the prior year. Annually thereafter, Syngenta must provide EPA any substantive changes to its grower education activities as part of the overall IRM compliance assurance program report. Syngenta must either submit a separate report or contribute to the report from the industry working group, ABSTC.

3. Syngenta must design and implement an ongoing IRM compliance assurance program designed to evaluate the extent to which growers purchasing *Bt*11 x MIR162 x MIR604 corn are complying with the IRM program and that takes such actions as are reasonably needed to assure that growers who have not complied with the program either do so in the future or lose their access to *Bt*11 x MIR162 x MIR604 corn. Syngenta shall coordinate with other *Bt* corn registrants in designing and implementing its compliance assurance program and integrate this registration into the current compliance assurance program used for their other *Bt* corn PIPs. Syngenta must prepare and submit within 90 days of the date of registration a written description of the compliance assurance program. Other required features of the program are described in paragraphs 5–15 below.

4. Syngenta must establish and publicize a "phased compliance approach," i.e., a guidance document that indicates how they will address instances of non-compliance with the terms of the IRM program and general criteria for choosing among options for responding to any non-compliant growers. While recognizing that for reasons of difference in business practices there are needs for flexibility between different companies, Syngenta must use a consistent set of standards for responding to non-compliance. The options shall include withdrawal of the right to purchase *Bt*11 x MIR162 x MIR604 corn for an individual grower or for all growers in a specific region. An individual grower found to be significantly out of compliance two years in a row would be denied sales of *Bt*11 x MIR162 x MIR604 corn the next year. Similarly, seed dealers who are not fulfilling their obligations to inform/educate growers of their IRM obligations will lose their opportunity to sell *Bt*11 x MIR162 x MIR604 corn.

5. The IRM compliance assurance program shall include an annual survey, conducted by an independent third party, of a statistically representative sample of growers of *Bt*11 x MIR162 x MIR604 corn who plant the vast majority of all corn in the United States and in areas in which the selection intensity is the greatest. The survey shall consider only those growers who plant 200 or more acres of corn in the Corn Belt or who plant 100 or more acres of corn in corn-cotton growing areas. The survey shall measure the degree of compliance with the IRM program by growers in different regions of the country and consider the potential impact of non-response.

The sample size and geographical resolution may be adjusted annually, based upon input from independent marketing research firms and academic scientists, to allow analysis of compliance behavior within regions or between regions. The sample size must provide a reasonable sensitivity for comparing results across the United States.

6. The survey shall be designed to provide an understanding of any difficulties growers encounter in implementing IRM requirements. An analysis of the survey results must include the reasons, extent, and potential biological significance of any implementation deviations.

7. The survey shall be designed to obtain grower feedback on the usefulness of specific educational tools and initiatives.

8. Syngenta shall provide a final written summary of the results of the prior year's survey (together with a description of the regions, the methodology used, and the supporting data) to EPA by January 31st of each year, beginning with 2010. Syngenta shall confer with other registrants and EPA on the design and content of the survey prior to its implementation.

9. Annually, Syngenta shall revise, and expand as necessary, its compliance assurance program to take into account the information collected through the compliance survey (required under paragraphs 6 through 8) and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high. Syngenta must confer with the Agency prior to adopting any changes.

10. Syngenta shall conduct an annual on-farm assessment program. Syngenta shall train its representatives who make on-farm visits with growers of *Bt*11 x MIR162 x MIR604 corn to perform assessments of compliance with IRM requirements. There is no minimum corn acreage size for this program. Therefore, growers will be selected for this program from across all farm sizes. In the event that any of these visits result in the identification of a grower who is not in compliance with the IRM program, Syngenta shall take appropriate action, consistent with its "phased compliance approach" to promote compliance.

11. Syngenta shall carry out a program for investigating legitimate "tips and complaints" that its growers are not in compliance with

the IRM program. Whenever an investigation results in the identification of a grower who is not in compliance with the IRM program, Syngenta shall take appropriate action, consistent with its "phased compliance approach."

12. If a grower, who purchases *Bt*11 x MIR162 x MIR604 corn for planting, was specifically identified as not being in compliance during the previous year, Syngenta shall visit with the grower and evaluate whether that the grower is in compliance with the IRM program for the current year.

13. Beginning January 31, 2010 and annually thereafter, Syngenta shall provide a report to EPA summarizing the activities carried out under their compliance assurance program for the prior year and the plans for the compliance assurance program during the current year. The report will include information regarding grower interactions (including, but not limited to, on-farm visits, verified tips and complaints, grower meetings and letters), the extent of non-compliance, corrective measures to address the non-compliance, and any follow-up actions taken. Syngenta may elect to coordinate information with other registrants and report collectively the results of compliance assurance programs.

14. Syngenta and the seed corn dealers for Syngenta must allow a review of the compliance records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including the names, personal information, and license number of the growers will be protected.

k. **Insect Resistance Monitoring and Remedial Action Plans for *Bt*11 x MIR162 x MIR604 Corn**

0. The Agency is imposing the following conditions for the Cry1Ab toxin expressed in *Bt*11 corn:

Syngenta will monitor for resistance to Cry1Ab expressed in Bt11 x MIR162 x MIR604 corn. The monitoring program shall consist of two approaches: (i) focused population sampling and laboratory testing and (ii) investigation of reports of less-than expected control of labeled insects. Should field-relevant resistance be confirmed, an appropriate resistance management action plan will be implemented.

i. *Focused Population Sampling*

Syngenta shall annually sample and bioassay populations of the key target pests: *Ostrinia nubilalis* (European corn borer; ECB), *Diatraea grandiosella* (Southwestern corn borer; SWCB), and *Helicoverpa zea* (corn earworm; CEW). Sampling for the target pests will be focused in areas identified as those with the highest risk of resistance development (e.g., where lepidopteran-active *Bt* hybrids are planted on a high proportion of the corn acres, and where the insect species are regarded as key pests of corn). Bioassay methods must be appropriate for the goal of detecting field-relevant shifts in population response to *Bt*11 x MIR162 x MIR604 corn and/or changes in resistance-allele frequency in response to the use of *Bt*11 x MIR162 x MIR604 corn and, as far as possible, should be consistent across sampling years to enable comparisons with historical data.

The number of populations to be collected shall reflect the regional importance of the insect species as a pest, and specific collection regions will be identified for each pest. For ECB, a minimum of 12 populations across the sampling region will be targeted for collection at each annual sampling. For SWCB, the target will be a minimum of six populations. For CEW, the target will be a minimum of ten populations. Pest populations should be collected from multiple corn-growing states reflective of different geographies and agronomic conditions. To obtain sufficient sensitivity to detect resistance alleles before they become common enough to cause measurable field damage, each population collection shall attempt to target 400 insect genomes (egg masses, larvae, mated females, and/or mixed-sex adults), but a successful population collection will contain a minimum of 100 genomes. It is recognized that it may not be possible to collect the target number of insect populations or genomes due to factors such as natural fluctuations in pest density, environmental conditions, and area-wide pest suppression.

The sampling program and geographic range of collections may be modified as appropriate based on changes in pest importance and for the adoption levels of *Bt*11 x MIR162 x MIR604 corn. The Agency shall be consulted prior to the implementation of such modifications.

Syngenta will report to the Agency by August 31st of each year, beginning in 2010, the results of the population sampling and bioassay monitoring program.

Any incidence of unusually low sensitivity to the Cry1Ab protein in bioassays shall be investigated as soon as possible to understand any field relevance of such a finding. Such investigations shall proceed in a stepwise manner until the field relevance can be either confirmed or refuted, and results of these shall be reported to the Agency annually before August 31st, beginning in 2010. The investigative steps will include:

1. Re-test progeny of the collected population to determine whether the unusual bioassay response is reproducible and heritable. If it is not reproducible and heritable, no further action is required.

2. If the unusual response is reproducible and heritable, progeny of insects that survive the diagnostic concentration will be tested using methods that are representative of exposure to *Bt*11 x MIR162 x MIR604 corn under field conditions. If progeny do not survive to adulthood, any suspected resistance is not field relevant and no further action is required.

3. If insects survive steps 1 and 2, resistance is confirmed, and further steps will be taken to evaluate the resistance. These steps may include:

    - determining the nature of the resistance (i.e., recessive or dominant, and the level of functional dominance);

- estimating the resistance-allele frequency in the original population;

- determining whether the resistance-allele frequency is increasing by analyzing field collections in subsequent years sampled from the same site where the resistance allele(s) was originally collected;

- determining the geographic distribution of the resistance allele by analyzing field collections in subsequent years from sites surrounding the site where the resistance allele(s) was originally collected.

Should field-relevant resistance be confirmed, and the resistance appears to be increasing or spreading, Syngenta will consult with the Agency to develop and implement a case- specific resistance management action plan.

ii. *Investigation of Reports of Unexpected Levels of Damage by the Target Pests*

Syngenta will follow up on grower, extension specialist or consultant reports of unexpected levels of damage by the lepidopteran pests listed on the pesticide label. Syngenta will instruct its customers to contact them if such incidents occur. Syngenta will investigate all legitimate reports submitted to the company or the company's representatives.

If reports of unexpected levels of damage lead to the suspicion of resistance in any of the key target pests (ECB, SWCB, and CEW), Syngenta will implement the actions described below, based on the following definitions of *suspected resistance* and *confirmed resistance*.

Suspected resistance

EPA defines *suspected resistance* to mean field reports of unexpected levels of insect feeding damage for which:

- the corn in question has been confirmed to be lepidopteran-active *Bt* corn;

- the seed used had the proper percentage of corn expressing *Bt* protein;

- the relevant plant tissues are expressing the expected level of *Bt* protein; and

- it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that there could be no other reasonable causes for the damage.

If resistance is suspected, Syngenta will instruct growers to do the following:

- Use alternative control measures in Bt11 x MIR162 x MIR604 corn fields in the affected region to control the target pest during the immediate growing season.

- Destroy *Bt*11 x MIR162 x MIR604 corn crop residues in the affected region within one month after harvest with a technique appropriate for local production practices to minimize the possibility of resistant insects over-wintering and contributing to the next season's target pest population.

Additionally, if possible, and prior to the application of alternative control measures or destruction of crop residue, Syngenta will collect samples of the insect population in the affected fields for laboratory rearing and testing. Such rearing and testing shall be conducted as expeditiously as practical.

Confirmed resistance

EPA defines *confirmed resistance* to mean, in the case of field reports of unexpected levels of damage from the key target pests, that all the following criteria are met:

- There is >30% insect survival and commensurate insect feeding in a bioassay, initiated with neonate larvae, that uses methods that are representative of exposure to Bt corn hybrids under field conditions (ECB and SWCB only).

- In standardized laboratory bioassays using diagnostic concentrations of the Bt protein suited to the target pest in question, the pest exhibits resistance that has a genetic basis and the level of survivorship indicates that there may be a resistance-allele frequency of = 0.1 in the sampled population.

- In standardized laboratory bioassays, the LC_{50} exceeds the upper limit of the 95% confidence interval of the LC_{50} for susceptible populations surveyed both in the original baselines developed for this pest species and in previous years of field monitoring

Response to Confirmed Resistance in a Key Target Pest as the Cause of Unexpected Levels of Damage in the Field

When field resistance is *confirmed* (as defined above), the following steps will be taken by Syngenta:

- EPA will receive notification within 30 days of resistance confirmation;

- Affected customers and extension agents will be notified about confirmed resistance within 30 days;

- Monitoring will be increased in the affected area and local target pest populations will be sampled annually to determine the extent and impact of resistance;

- If appropriate (depending on the resistant pest species, the extent of resistance, the timing of

resistance, and the nature of resistance, and the availability of suitable alternative control measures), alternative control measures will be employed to reduce or control target pest populations in the affected area. Alternative control measures may include advising customers and extension agents in the affected area to incorporate crop residues into the soil following harvest to minimize the possibility of over-wintering insects, and/or applications of chemical insecticides;

- Unless otherwise agreed with EPA, stop sale and distribution of the relevant lepidopteran-active *Bt* corn hybrids in the affected area immediately until an effective local mitigation plan approved by EPA has been implemented;

- Syngenta will develop a case-specific resistance management action plan within 90 days according to the characteristics of the resistance event and local agronomic needs. Syngenta will consult with appropriate stakeholders in the development of the action plan, and the details of such a plan shall be approved by EPA prior to implementation;

- Notify affected parties (e.g., growers, consultants, extension agents, seed distributors, university cooperators and state/federal authorities as appropriate) in the region of the resistance situation and approved action plan; and

- In subsequent growing seasons, maintain sales suspension and alternative resistance management strategies in the affected region(s) for the *Bt* corn hybrids that are affected by the resistant population until an EPA-approved local resistance management plan is in place to mitigate the resistance.

A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by August 31st each year, beginning in 2010, for the duration of the conditional registration.

1. The Agency is imposing the following conditions for the Vip3Aa20 toxin expressed in MIR162 maize:

A detailed resistance monitoring program and final remedial action plan, integrating standard procedures developed by the ABSTC (as outlined below) and harmonized with resistance monitoring programs and remedial action plans established for previously registered Syngenta Bt corn products, for the key target pests of CEW and SWCB must be submitted within 90 days of the date of registration.

Syngenta will monitor for resistance to Vip3Aa20 expressed in *Bt*11 x MIR162 x MIR604 corn. The monitoring program shall consist of two approaches: (i) focused population sampling and laboratory testing and (ii) investigation of reports of less-than expected control of labeled insects. Should field-relevant resistance be confirmed, an appropriate resistance management action plan will be implemented.

Focused Population Sampling

Syngenta shall annually sample and bioassay populations of the key target pests: *Diatraea grandiosella* (Southwestern corn borer; SWCB) and *Helicoverpa zea* (corn earworm; CEW). Sampling for the target pests will be focused in areas identified as those with the highest risk of resistance development (e.g., where lepidopteran-active Bt hybrids are planted on a high proportion of the corn acres, and where the insect species are regarded as key pests of corn). Bioassay methods must be appropriate for the goal of detecting field- relevant shifts in population response to Bt11 x MIR162 x MIR604 corn and/or changes in resistance-allele frequency in response to the use of *Bt*11 x MIR162 x MIR604 corn and, as far as possible, should be consistent across sampling years to enable comparisons with historical data.

The sampling program and geographic range of collections may be modified as appropriate based on changes in pest importance and for the adoption levels of Bt11 x MIR162 x MIR604 corn. The Agency shall be consulted prior to the implementation of such modifications

Syngenta will report to the Agency by August 31st of each year, beginning in 2010, the results of the population sampling and bioassay monitoring program.

Any incidence of unusually low sensitivity to the Vip3Aa20 protein in bioassays shall be investigated as soon as possible to understand any field relevance of such a finding. Such investigations shall proceed in a stepwise manner until the field relevance can be either confirmed or refuted, and results of these shall be reported to the Agency annually before August 31st, beginning in 2010. The investigative steps will include:

0. Re-test progeny of the collected population to determine whether the unusual bioassay response is reproducible and heritable. If it is not reproducible and heritable, no further action is required.

1. If the unusual response is reproducible and heritable, progeny of insects that survive the diagnostic concentration will be tested using methods that are representative of exposure to Bt11 x MIR162 x MIR604 corn under field conditions. If progeny do not survive to adulthood, any suspected resistance is not field relevant and no further action is required.

2. If insects survive steps 1 and 2, resistance is confirmed, and further steps will be taken to evaluate the resistance. These steps may include:

    - determining the nature of the resistance (i.e., recessive or dominant, and the level of functional dominance);

    - estimating the resistance-allele frequency in the original population;

    - determining whether the resistance-allele frequency is increasing by analyzing field

collections in subsequent years sampled from the same site where the resistance allele(s) was originally collected;

- determining the geographic distribution of the resistance allele by analyzing field collections in subsequent years from sites surrounding the site where the resistance allele(s) was originally collected.

Investigation of Reports of Unexpected Levels of Damage by the Target Pests

Syngenta will follow up on grower, extension specialist or consultant reports of unexpected levels of damage by the lepidopteran pests listed on the pesticide label. Syngenta will instruct its customers to contact them if such incidents occur. Syngenta will investigate all legitimate reports submitted to the company or the company's representatives.

If reports of unexpected levels of damage lead to the suspicion of resistance in any of the key target pests (SWCB and CEW), Syngenta will implement the actions described below, based on the following definitions of *suspected resistance* and *confirmed resistance*.

Suspected resistance

EPA defines *suspected resistance* to mean field reports of unexpected levels of insect feeding damage for which:

- the corn in question has been confirmed to be lepidopteran-active *Bt* corn;

- the seed used had the proper percentage of corn expressing *Bt* protein;

- the relevant plant tissues are expressing the expected level of *Bt* protein; and

- it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that there could be no other reasonable causes for the damage.

The Agency does not interpret suspected resistance to mean grower reports of possible control failures or suspicious results from annual insect monitoring assays, nor does the Agency intend that extensive field studies and testing be undertaken to confirm scientifically the presence of insects resistant to Bt11 x MIR162 x MIR604 corn in commercial production fields before responsive measures are undertaken.

If resistance is *suspected*, Syngenta will instruct growers to do the following:

- Use alternative control measures in Bt11 x MIR162 x MIR604 corn fields in the affected region to control the target pest during the immediate growing season.

- Destroy Bt11 x MIR162 x MIR604 corn crop residues in the affected region within one month after harvest with a technique appropriate for local production practices to minimize the possibility of resistant insects over-wintering and contributing to the next season's target pest population.

Additionally, if possible, and prior to the application of alternative control measures or destruction of crop residue, Syngenta will collect samples of the insect population in the affected fields for laboratory rearing and testing. Such rearing and testing shall be conducted as expeditiously as practical.

Confirmed resistance

EPA defines confirmed resistance to mean, in the case of field reports of unexpected levels of damage from the key target pests, that all the following criteria are met:

- There is >30% insect survival and commensurate insect feeding in a bioassay, initiated with neonate

larvae, that uses methods that are representative of exposure to Bt corn hybrids under field conditions (SWCB only).

- In standardized laboratory bioassays using diagnostic concentrations of the Bt protein suited to the target pest in question, the pest exhibits resistance that has a genetic basis and the level of survivorship indicates that there may be a resistance-allele frequency of = 0.1 in the sampled population.

- In standardized laboratory bioassays, the LC_{50} exceeds the upper limit of the 95% confidence interval of the LC_{50} for susceptible populations surveyed both in the original baselines developed for this pest species and in previous years of field monitoring.

Response to Confirmed Resistance in a Key Target Pest as the Cause of Unexpected Levels of Damage in the Field

When field resistance is confirmed (as defined above), the following steps will be taken by Syngenta:

- EPA will receive notification within 30 days of resistance confirmation;

- Affected customers and extension agents will be notified about confirmed resistance within 30 days;

- Monitoring will be increased in the affected area and local target pest populations will be sampled annually to determine the extent and impact of resistance;

- If appropriate (depending on the resistant pest species, the extent of resistance, the timing of resistance, and the nature of resistance, and the availability of suitable alternative control measures), alternative control measures will be employed to reduce or control target pest populations in the affected area. Alternative control

measures may include advising customers and extension agents in the affected area to incorporate crop residues into the soil following harvest to minimize the possibility of over-wintering insects, and/or applications of chemical insecticides;

- Unless otherwise agreed with EPA, stop sale and distribution of the relevant lepidopteran-active *Bt* corn hybrids in the affected area immediately until an effective local mitigation plan approved by EPA has been implemented;

- Syngenta will develop a case-specific resistance management action plan within 90 days according to the characteristics of the resistance event and local agronomic needs. Syngenta will consult with appropriate stakeholders in the development of the action plan, and the details of such a plan shall be approved by EPA prior to implementation;

- Notify affected parties (e.g., growers, consultants, extension agents, seed distributors, university cooperators and state/federal authorities as appropriate) in the region of the resistance situation and approved action plan; and

- In subsequent growing seasons, maintain sales suspension and alternative resistance management strategies in the affected region(s) for the *Bt* corn hybrids that are affected by the resistant population until an EPA-approved local resistance management plan is in place to mitigate the resistance.

2. The Agency is imposing the following conditions for the mCry3A toxin expressed in MIR604 corn:

   A revised mCry3A monitoring plan that incorporates Bt11 x MIR162 x MIR604 corn must be submitted to the Agency within 90 days of the date of registration. Syngenta must monitor for mCry3A resistance and/or trends in increased tolerance for corn rootworm. Sampling should be focused in those areas in which there is the highest risk of resistance development. In addition to

mortality assays, consider utilizing sublethal bioassays (e.g., head capsule measurements) and molecular marker methods for corn rootworm monitoring.

By January 31, 2010, submit data generated by the following actions: (a) initiate establishment of CRW strains that are resistant to mCry3A and investigate the nature, inheritance, and fitness costs of specific mechanisms of resistance to mCry3A, (b) study the behavioral deterrence (avoidance) mechanism further, and (c) continue studies on the biological impact of CRW adults surviving on corn expressing the mCry3A toxin.

Syngenta must develop and validate an appropriate discriminating or diagnostic dose assay by January 31, 2010.

Syngenta must finalize rootworm damage guidelines and submit these to EPA by January 31, 2010.

Syngenta must follow-up on grower, extension specialist or consultant reports of unexpected damage or control failures for corn rootworm.

Syngenta must provide EPA with an annual resistance monitoring report by August 31st each year, beginning in 2010, reporting on populations collected the previous year.

The following program summary describes, in order or events, the steps that must be taken to implement a remedial action plan if resistance to corn rootworm is confirmed (this general process has been implemented for other lepidopteran and corn rootworm Bt corn products).

0. *Definition of Suspected Resistance*. Resistance will be suspected if investigations of of unexpected damage reports show that:
    - implicated maize plant roots were expressing the mCry3A protein at the expected level;

- alternative causes of damage or lodging, such as non-target pest insect species, weather, physical damage, larval movement from alternate hosts, planting errors, and other reasonable causes for the observations, have been ruled out;

- the level of damage exceeds guidelines for expected damage.

If resistance is "suspected," Syngenta will instruct affected growers to use alternate pest control measures such as adulticide treatment, crop rotation the following year, or use of soil or seed insecticides the following year. These measures are intended to reduce the possibility of potentially resistant insects contributing to the following year's pest population.

1. *Confirmation of Resistance*. Resistance will be confirmed if all of the following criteria are met by progeny from the target pest species sampled from the area of "suspected resistance":

   - the proportion of larvae that can feed and survive on mCry3A roots from neonate to adult is significantly higher than the baseline proportion (currently being established);

   - the LC_{50} of the test population exceeds the upper limit of the 95% confidence interval for the LC_{50} of a standard unselected population and/or survival in the diagnostic assay is significantly greater than that of a standard unselected population, as established by the ongoing baseline monitoring program;

   - the ability to survive is heritable;

- mCry3A plant assays determine that damage caused by surviving insects would exceed economic thresholds; and

- the identified frequency of field resistance could lead to widespread product failure if subsequent collections in the affected field area(s) demonstrated similar bioassay results.

2. *Response to Confirmed Resistance*. When resistance is "confirmed," the following steps will be taken:

   - EPA will receive notification within 30 days of resistance confirmation;

   - affected customers and extension agents will be notified about confirmed resistance;

   - affected customers and extension agents will be encouraged to employ alternative corn rootworm control measures;

   - sale and distribution of mCry3A corn in the affected area will cease immediately; and

   - a long-term resistance management action plan will be devised according to the characteristics of the resistance event and local agronomic needs. [The details of such a plan should be approved by approved by EPA and all appropriate stakeholders.]

l. **Annual Reporting Requirements for Bt11 x MIR162 x MIR604 Corn**

0. Annual Sales: reported and summed by state (county level data available by request) January 31st each year, beginning in 2010;

1. Grower Agreements: number of units of Bt11 x MIR162 x MIR604 corn seed shipped or sold and not returned, and the number of such units that were sold to persons who have signed grower agreements, January 31st each year, beginning in 2010;

2. Grower Education: substantive changes to education program completed previous year, January 31st each year, beginning in 2010;

3. Compliance Assurance Program: compliance assurance program activities and results for the prior year and plans for the compliance assurance program for the current year, January 31st each year, beginning in 2010;

4. Compliance Survey Results: results of annual surveys for the prior year and survey plans for the current year; full report January 31st each year, beginning in 2010;

5. Insect Resistance Monitoring Results: results of monitoring and investigations of damage reports, August 31st each year, beginning in 2010.

I. Additional Contact Information

Ombudsman, Biopesticides and Pollution Prevention Division (7511P)
Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460