A global approach to resistance monitoring

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Abstract

Transgenic crops producing insecticidal toxins from the bacterium Bacillus thuringiensis (Bt) have been grown in many parts of the world since 1996. In the United States, the Environmental Protection Agency (EPA) has required that industry submit insect resistance management (IRM) plans for each Bt corn and cotton product commercialized. A coalition of stakeholders including the EPA, USDA, academic scientists, industry, and grower organizations have cooperated in developing specific IRM strategies. Resistance monitoring (requiring submission of annual reports to the EPA), and a remedial action plan addressing any contingency if resistance should occur, are important elements of these strategies. At a global level, Monsanto conducts baseline susceptibility studies (prior to commercialization), followed by monitoring studies on target pest populations, for all of its commercialized Bt crop products. To date, Monsanto has conducted baseline/monitoring studies in Argentina, Australia, Brazil, Canada, China, Colombia, India, Mexico, the Philippines, South Africa, Spain, and the United States. Examples of pests on which resistance monitoring has been conducted include cotton bollworm, Helicoverpa zea, European corn borer, Ostrinia nubilalis, pink bollworm, Pectinophora gossypiella, Southwestern corn borer, Diatraea grandiosella, tobacco budworm, Heliothis virescens, and western corn rootworm, Diabrotica virgifera virgifera, in the United States, cotton bollworm, Helicoverpa armigera, in China, India and Australia, and H. virescens and H. zea in Mexico. No field-selected resistance to Bt crops has been documented.

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1. Introduction

Cotton and corn expressing Bacillus thuringiensis (Bt) Cry1Ac and Cry1Ab δ-endotoxins, respectively, for the control of key lepidopteran pests have been commercially available in multiple varieties since 1996 (for example, Bollgard® cotton and Yieldgard® corn, Monsanto Co). Bollgard® and Yieldgard® provide excellent control of several economically important pest species, including the European corn borer (Ostrinia nubilalis), tobacco budworm Heliothis virescens (F.) and pink bollworm Pectinophora gossypiella (Saunders). As a consequence, these products have been widely adopted in many counties spanning North and South America, Asia, Africa, Australia, and Europe, effectively reducing the use of synthetic insecticides in cotton and corn systems in these countries (Brookes and Barfoot, 2006; Fernandez-Cornejo and Caswell, 2006).

While the adoption of these products has significantly reduced pesticide use for lepidopteran pests in cotton and corn, these first generation products have not provided complete protection against all major lepidopteran pests because several species (particularly noctuids in the genera Helicoverpa and Spodoptera) are inherently less susceptible to Cry1Ab and Cry1Ac (MacIntosh et al., 1990). Bollgard II® cotton expressing both Cry1Ac and Cry2Ab2 was developed in an attempt to increase efficacy, expand the host spectrum, and delay the evolution of Bt resistance in lepidopteran cotton pests (Dankocsik et al., 1990; Perlak et al., 2001; Greenplate et al., 2003). Bollgard II® cotton has been commercially available since 2003, and has provided increased efficacy against the budworm/bollworm complex, and enhanced activity against beet armyworm, Spodoptera exigua (Hübner), fall armyworm, Spodoptera...
2. IRM for Bt Crops

Good stewardship of transgenic crop products requires the generation of accurate and reliable data on the products, complying with all relevant regulations, ensuring that products are safe and are used in an environmentally responsible manner, and promoting the proper use of products by all licensees. In the United States, the Environmental Protection Agency (EPA) requires that industry submit insect resistance management (IRM) plans for each Bt corn and Bt cotton product commercialized. A coalition of stakeholders, including the EPA, USDA, academic scientists, industry, and grower organizations, have cooperated in developing specific IRM strategies. Resistance monitoring, requiring submission of annual reports to the EPA, and a remedial action plan, addressing any contingency if resistance should occur, are important elements of these strategies.

Prior to widespread commercialization of the product, it is necessary to develop baseline data on susceptibility of target pest populations from appropriate geographical areas to the Bt protein(s) contained in the Bt crop. These data provide insight into the natural variability among pest populations in the geographical range of adoption and, more importantly, can be used to assess future shifts in susceptibility to the proteins in the transgenic crops. Subsequent routine monitoring is carried out annually for each target pest with cooperation from government and academic laboratories. At a global level, Monsanto conducts baseline susceptibility studies (prior to commercialization), followed by monitoring studies on target pest populations, for all of its commercialized Bt crop products. To date, Monsanto has conducted baseline/monitoring studies in Argentina, Australia, Brazil, Canada, China, Colombia, India, Mexico, the Philippines, South Africa, Spain, and the United States. Examples of pests on which resistance monitoring has been conducted include cotton bollworm, Helicoverpa zea, European corn borer, O. nubilalis, pink bollworm, P. gossypiella, Southwestern corn borer, Diatraea grandiosella, tobacco budworm, H. virescens, and western corn rootworm, Diabrotica virgifera virgifera, in the United States, cotton bollworm, Helicoverpa armigera, in China, India and Australia, and H. virescens and H. zea in Mexico. In the US, Cry1Ac and Cry2Ab2 monitoring of Bt cotton pests is conducted by the USDA in Stonewall, MS (H. virescens), and by academic scientists at the University of Arkansas (H. zea) and the University of Arizona (P. gossypiella). Cry1Ab and Cry1F monitoring for Bt corn pests is conducted by scientists at the University of Nebraska (O. nubilalis) and University of Missouri (D. grandiosella), and by a contract laboratory sponsored by an industry consortium (H. zea).

3. Key elements of resistance monitoring programs

In all of Bt resistance monitoring programs in the US, coordinated efforts are in place to collect insect populations from areas of high Bt adoption and high pest abundance. Each collection is intended to be large enough to be representative of the local field population and to provide a reasonable opportunity to detect any resistant alleles that may be present in that population. Enough collections are made to capture the natural variation prevalent in the region. The collections are transferred in a timely manner to the monitoring laboratories. Limitations encountered in these routine monitoring programs include logistical issues which can preclude collection of representative populations and the use of phenotypic screens which have a limited ability to detect fully recessive resistant alleles. In Australia, single family, mated pairs have been used in resistance monitoring programs to address some of the limitations associated with other methods, but this approach is not widely used in resistance monitoring laboratories because of the time and resources involved and because fully recessive resistant alleles are less of a concern in IRM than partially dominant alleles.

Establishing and testing of insect populations in the laboratory is extremely labor-intensive and requires dedicated personnel. Adapting field-collected insects to laboratory rearing condition (on diet), fecundity reduction in the laboratory, isolation of individual populations, and transfer of diseases from the field to the laboratory are just a few of the challenges faced by monitoring laboratories. Rearing insects to the F1 and F2 generation is routinely required for testing populations and additional rearing often is necessary if the initial collections are small, though testing early generations is recommended. A good understanding of the biology of the target insects is critical to the production of quality insects for use in bioassays. It is also very important that a susceptible laboratory colony is available for comparisons across years and regions.

Industry provides the Bt proteins required by researchers responsible for the monitoring programs in the US as well as globally (for example, Monsanto supports programs related to YieldGard® and Bollgard®). Some of the proteins currently being tested are Cry1Ac and Cry2Ab2 (as found in Bollgard® II cotton) and Cry1Ab and Cry1F (found in Bt corn products). MVP II lyophilized powder (expressing 19.17% Cry1Ac), corn leaf powder expressing Cry2Ab2, and purified Cry1Ab and Cry1F protein are sources of proteins which are used in the baseline/monitoring programs. MVP II and corn powder expressing Cry2Ab2 are used instead of purified protein because of their stability (relative to the purified protein), the ease of transfer of protein supplies globally, and the lower cost compared to the generation of purified protein. When new protein sources, or new batches from the same source, must be introduced into a monitoring program, bridging studies always are conducted using well characterized standards in replicated bioassays (with a sensitive...
insect) and ELISA. Tracking and provision of a reliable protein source is extremely important in interpreting data across laboratories in different countries and making valid comparisons over time.

Both diet incorporation and diet overlay bioassays are used by monitoring laboratories. Although diet incorporation is a more robust assay type, diet overlay methodology is simpler and requires less protein, which is useful when screening insects that are less sensitive to the \textit{Bt} protein in question. The response of insects to \textit{Bt} proteins can be assessed using mortality criteria and/or developmental arrest. In many instances, molt inhibition to the second instar, developmental arrest below the third instar, and mass of surviving insects is recorded to assess toxicity responses. The choice of an appropriate endpoint depends on the assay conditions, the biology of the insect species being studied, and the length of the assay. Results generated are subjected to an appropriate statistical test to determine the presence or absence of resistant populations.

In addition, field performance and any signs of unexpected damage are tracked with the assistance of extension agents, consultants and growers (reactive monitoring). In fields where unexpectedly high damage levels are witnessed, surviving larvae are collected and transported to the appropriate monitoring facility for more complete characterization of the susceptibility profile of the population. In addition, appropriate tissue samples from the damaged crop plants (and controls) are shipped to Monsanto Company headquarters located in St. Louis, MO, USA to determine protein expression and to detect of the presence of conventional plants in \textit{Bt} crop fields.

4. Conclusions

Resistance monitoring is an integral part of IRM for \textit{Bt} crops. Resistance monitoring programs have been implemented wherever \textit{Bt} crops have been commercialized, using approaches and materials which have been globally standardized. No field-selected resistance to \textit{Bt} crops has been documented in any of these programs despite intensive sampling. The successful creation of resistance monitoring programs to support the global use of \textit{Bt} crops reflects the importance that the scientific community and product developers like Monsanto place upon ensuring the durability of \textit{Bt} crops through responsible stewardship.

References


