Insects Find Crack In Biotech Corn's Armor

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by DAN CHARLES

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Scientists say the corn rootworm is growing resistant to Bt corn.

Hidden in the soil of Illinois and Iowa, a new generation of insect larvae appears to be munching happily on the roots of genetically engineered corn, according to scientists. It's bad news for corn farmers, who paid extra money for this line of corn, counting on the power of its inserted genes to kill those pests. It's also bad news for the biotech company Monsanto, which inserted the larvae-killing gene in the first place.

In fact, the gene's apparent failure, as reported in the journal *PLoS One*, may be the most serious threat to a genetically modified crop in the U.S. since farmers first started growing them 15 years ago. The economic impact could be "huge," says the University of Arizona's Bruce Tabashnik, one of the country's top experts on the adaptation of insects to genetically

engineered crops. Billions of dollars are at stake.

The story of how this happened is long and complicated, but the details are important, so let's start at the beginning.

Almost the entire agricultural biotech industry has been built on just two genetic traits, and our story involves one of them.

The gene (actually a family of genes) in this story — the first pillar of the industry — was copied from an insect-killing bacterium called *Bacillus thuringiensis*, or Bt. In the 1980s, scientists managed to insert a Bt gene into plants, and voila, the plant cells started manufacturing the same worm-killing toxin as the bacteria. (The other big gene for the agricultural biotech industry allows a plant to survive doses of the popular herbicide glyphosate, widely known by Monsanto's trade name, Roundup.)

So-called Bt corn went on sale in the late 1990s. It has been astonishingly effective against the European corn borer, a common pest.

But from the beginning, scientists worried that biotech companies were overusing Bt and increasing the chances that it would eventually stop working. Why? The key word is resistance.

The more widely you spray any insecticide, the more likely you are to uncover and promote the growth of a new strain of insects that's resistant to your insect killer. It has happened with one insecticide after another over the decades. Eventually, scientists said, the same thing would happen to a crop that carries its own insecticide. Covering fields with Bt crops would lead to a strain of insects that the crops didn't kill.

So university researchers and federal regulators came up with a strategy to preserve Bt's effectiveness. First of all, they said Bt crops (mainly corn and cotton) should be extremely effective. Ideally, they would kill 99.99 percent of all the target insects that fed on them.

And for those rare insects that survived, regulators came up with a second line of defense, to prevent resistant insects from mating and producing lots of resistant offspring. Farmers who grew Bt corn (or cotton) were required to grow non-Bt crops on some of their farm, as a "refuge" for normal insects. That way, the rare, surviving, resistant insects would probably find non-resistant mates, instead of each other, and their offspring still would (likely) be killed by the Bt corn.

To the surprise of some environmentalists, the strategy has worked. There's no evidence that the European corn borer has evolved resistance to the Bt toxin. The same goes for some insects that feed on cotton, such as the pink bollworm — at least

in the United States.

Yet the danger is real, as shown by something that happened in India. Tabashnik says farmers there ignored rules that required them to plant refuges of non-Bt cotton. As feared, a resistant strain of pink bollworm emerged in 2009 to prey on cotton fields.

Here in the U.S., though, the Bt train rolled on. After its success against the European corn borer and various cotton pests, scientists created corn plants that included another version of the Bt gene, one that produces a toxin that is lethal to larvae of some beetles, such as the corn rootworm. This insect is an important scourge of corn fields; at the time, farmers reportedly spent $1 billion a year on insecticides to fight it.

This new Bt gene, however, did not produce a "high dose" that killed 99.99 percent of all rootworms. Instead, it was what you'd call a "moderate dose," says Tabashnik. He says it's the worst situation: "The fastest way to get resistance."

So a scientific advisory panel urged the Environmental Protection Agency to strengthen the second line of defense against resistance, and demand large refuges on non-Bt corn. They proposed that farmers be allowed to plant Bt corn with this new gene on no more than half of their corn acres.

Monsanto argued that such a large refuge wasn't necessary, and the EPA agreed. In 2003, the agency decided to allow farmers to plant this new product on 80 percent of their corn acres.

The scientists who called for caution now are saying "I told you so," because there are signs that a new strain of resistant rootworms is emerging. In eastern Iowa, northwestern Illinois, and parts of Minnesota and Nebraska, rows of Bt corn have toppled over, their roots eaten by rootworms. Entomologist Aaron Gassmann at Iowa State University, who authored the *PLoS One* paper, collected insects from some of these fields and found many with a greater-than-expected ability to tolerate Bt.

Monsanto says other factors may be causing this. Because corn is so profitable right now, many farmers are growing corn year after year, causing a boom in insects that feed on corn.

But a committee of experts at the EPA is now recommending that biotech companies put into action, for the first time, a "remedial action plan" aimed at stopping the spread of such resistant insects. The agency's experts want farmers in areas where such damage has been observed to stop planting this kind of Bt corn altogether. Instead, those farmers will have to use other methods, such as spraying chemical insecticides, to control the rootworm. Some may simply plant soybeans or other crops instead.

The EPA's experts also are suggesting that the agency reconsider its approval of a new kind of rootworm-killing corn, which Monsanto calls SmartStax. This new version of Bt corn includes two different Bt genes that are supposed to kill the rootworm in different ways.

This should help prevent resistance from emerging, and the EPA is allowing farmers to plant it on up to 95 percent of their corn acres. But if one of those genes is already compromised, Tabashnik says, such a high percentage of Bt corn could rapidly produce insects that are resistant to the second one, too.

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Field-Evolved Resistance to Bt Maize by Western Corn Rootworm

Aaron J. Gassmann*, Jennifer L. Petzold-Maxwell, Ryan S. Keweshan, Mike W. Dunbar

Department of Entomology, Iowa State University, Ames, Iowa, United States of America

Abstract

Background: Crops engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt) are planted on millions of hectares annually, reducing the use of conventional insecticides and suppressing pests. However, the evolution of resistance could cut short these benefits. A primary pest targeted by Bt maize in the United States is the western corn rootworm *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae).

Methodology/Principal Findings: We report that fields identified by farmers as having severe rootworm feeding injury to Bt maize contained populations of western corn rootworm that displayed significantly higher survival on Cry3Bb1 maize in laboratory bioassays than did western corn rootworm from fields not associated with such feeding injury. In all cases, fields experiencing severe rootworm feeding contained Cry3Bb1 maize. Interviews with farmers indicated that Cry3Bb1 maize had been grown in those fields for at least three consecutive years. There was a significant positive correlation between the number of years Cry3Bb1 maize had been grown in a field and the survival of rootworm populations on Cry3Bb1 maize in bioassays. However, there was no significant correlation among populations for survival on Cry34/35Ab1 maize and Cry3Bb1 maize, suggesting a lack of cross resistance between these Bt toxins.

Conclusions/Significance: This is the first report of field-evolved resistance to a Bt toxin by the western corn rootworm and by any species of Coleoptera. Insufficient planting of refuges and non-recessive inheritance of resistance may have contributed to resistance. These results suggest that improvements in resistance management and a more integrated approach to the use of Bt crops may be necessary.

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* E-mail: aaronjg@iastate.edu

Introduction

Transgenic crops engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt) were planted on more than 58 million hectares worldwide in 2010 [1]. Benefits of Bt crops include reduced use of harmful insecticides and regional suppression of some key agricultural pests [2,3,4,5,6]. Within the United States, and worldwide, more area is planted to Bt maize *Zea mays* L. than any other Bt crop [1]. The western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is among the most serious pests of maize within the United States, with larval feeding on maize roots causing the majority of crop losses from this pest [7]. Beginning in 2003, Bt maize was commercialized for control of western corn rootworm larvae and was rapidly adopted by farmers, constituting over 45% of maize crop in the United States during 2009 [8,9]. However, the evolution of resistance by the western corn rootworm could cut short the benefits of Bt maize.

The refuge strategy is used in the United States and elsewhere to delay pest resistance to Bt crops [10]. This strategy uses non-Bt host plants as a refuge for Bt susceptible genotypes. Mating of homozygous susceptible pests with pests that are homozygous for Bt resistance produces progeny that are heterozygous for resistance traits. The delay in resistance expected under the refuge strategy becomes greater as the dominance of resistance decreases and is greatest when resistance is completely recessive [11]. Thus, as the area planted to refuge decreases or resistance becomes more dominant, pests are predicted to evolve resistance more quickly [11,12].

The western corn rootworm has repeatedly demonstrated its ability to adapt to pest management strategies [7]. Examples include the evolution of resistance to conventional insecticides and the cultural practice of crop rotation [13,14,15]. The widespread planting of Bt maize targeting western corn rootworm raised concerns that this pest would evolve resistance to Bt. Of additional concern are data suggesting that resistance of western corn rootworm to Bt maize is not recessive [16]. Furthermore, a lack of compliance in planting of refuges has been documented among farmers that grow Bt maize in the United States [17]. Both of these factors are expected to increase the risk of western corn rootworm evolving Bt resistance.