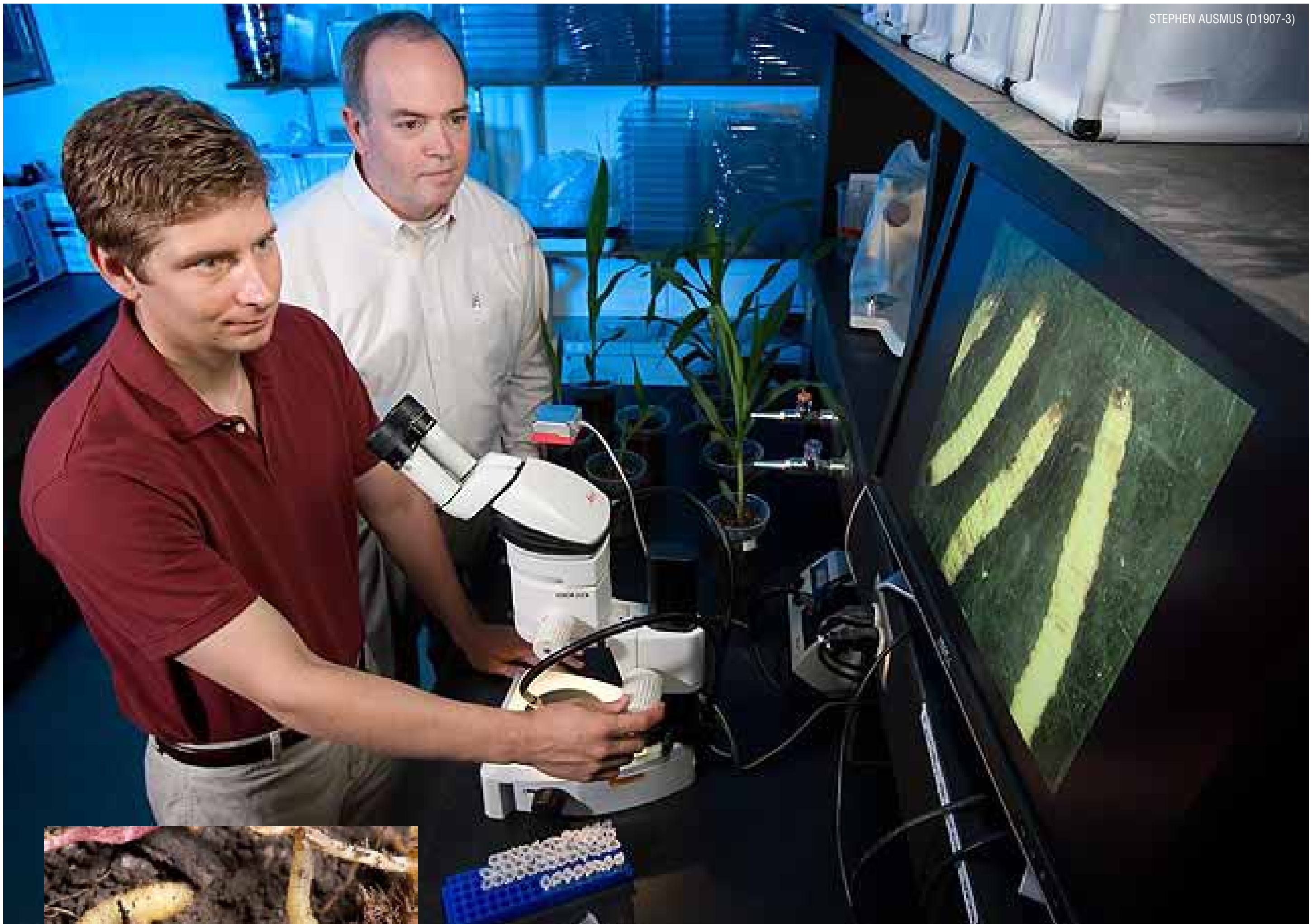
Rooting Out Rootworm Resistance



Above: Entomologists Aaron Gassmann (left), of Iowa State University, and Tom Sappington, with ARS, observe magnified western corn rootworm larvae to measure the size of their head capsules. Larvae exposed to *Bt* corn roots may grow more slowly than unexposed larvae, and head capsule width is an accurate indicator of larval growth stage. **Inset:** Larvae of western corn rootworm, *Diabrotica virgifera virgifera,* feeding on corn roots.

The western corn rootworm (WCR) is a highly destructive pest that has required more acres of conventional insecticide use per year than any other pest since it invaded the Midwest in the 1960s. Today, WCR continues to be the most important pest of corn production across the Midwest, in part because it has developed resistance to conventional crop rotation, which was once a highly effective management tactic.

Certain types of *Bt* corn, which have been genetically engineered to produce a particular insect-specific toxin protein derived from the bacterium *Bacillus thuringiensis,* are effective in the control of WCR. These products have partially replaced the use of conventional insecticides for WCR control. But laboratory tests strongly suggest that if *Bt* technology is not managed effectively in the field, WCR will acquire resistance to *Bt* over time.

Entomologists Bruce Hibbard, in the Plant Genetics Research Unit (PGRU) in Columbia, Missouri, and Tom Sappington, in the Corn Insects and Crop Genetics Research Unit (CICGRU) in Ames, Iowa, together with Blair Siegfried, at the University of Nebraska-Lincoln, and Aaron Gassmann, at Iowa State University, are analyzing the evolution of *Bt* resistance in WCR to help safeguard this technology for the future.

Some *Bt* corn lines targeting WCR produce a protein toxin known as "Cry3Bb1." Hibbard and collaborators recently documented that WCR exposed to these corn lines, at low to moderate doses under greenhouse conditions, developed *Bt* resistance after just three generations. This result was later validated in a controlled field test.

Several colonies of WCR that are resistant to Cry3Bb1 have now been established and are being used by Sappington's group to identify genetic markers associated with the resistance. Similar studies are being conducted with *Bt*-corn lines that produce mCry3A and other Cry proteins.

"Development of colonies resistant to distinct *Bt* products will allow us to evaluate the potential for cross-resistance in WCR," says Hibbard.

Rapid Resistance

As a strategy to control the evolution of *Bt* resistance in WCR populations, Hibbard and collaborators are currently testing the concept of using non-*Bt* plants as insect "refuges." This method promotes the mating of *Bt*-resistant insects with *Bt*-susceptible insects that emerge from refuge plants, which do not produce *Bt*. In this way, *Bt* susceptibility is maintained in the WCR population.

In 2006, Hibbard, along with CICGRU and University of Missouri collaborators, documented that without a refuge, WCR populations quickly develop *Bt* resistance under laboratory conditions. At the university's Bradford Research and Extension Center, the team evaluated *Bt*-resistant and *Bt*-susceptible (control) WCR colonies. *Bt* corn and non-*Bt* corn plants were infested with 500 viable rootworm eggs from the resistant or control colonies. The number of *Bt*-resistant insects recovered from *Bt* corn was 10 times greater from eggs taken from the *Bt*-resistant colony than from eggs taken from the control colony. Each colony did equally well on the non-*Bt* corn.

"This emphasizes the importance of farmers planting refuges, because resistance developed in the absence of a refuge," says Hibbard. Current work specifically tests the effectiveness of different refuge types.

Gathering Genetic Intelligence To Combat Western Corn Rootworm

At the Ames lab, DNA marker technology is being used to glean valuable genetic intelligence on the rootworms. DNA marker sequences are short stretches of DNA along the pest's genome that uniquely identify a region of that chromosome. A DNA marker that is located near a gene that confers a trait of interest can often be detected more easily than the gene itself.

Sappington's team is also using DNA markers to genetically locate the gene or genes responsible for "rotation resistance," an egg-laying behavior that enables some rootworm populations to survive rotations of corn with soybean, a nonhost crop on which the pest's caterpillars cannot feed.

Farmers in the Midwest have long used corn-soybean rotations, which allows them to forgo using soil insecticides on their corn. But starting in 1987, reports emerged from eastern Illinois that female rootworms were flying out of corn fields to deposit their eggs in nearby soybean crops—in effect, synchronizing their larval offsprings' emergence to the next season's rotational corn crop.

The trait has since spread to parts of Indiana, Ohio, Michigan, Missouri, Iowa, and Wisconsin and to Ontario, Canada. A DNA marker associated with rotation resistance would help alert farmers to when the trait has, or has not, spread to their region.

DNA markers are also being developed for examining genetic variation and gene flow within and between WCR populations.

"Though difficult to achieve, information on the population ecology and genetics of WCR are crucial for designing effective resistance-management strategies to maintain the viability of current control technologies, including *Bt* corn," says Craig Abel, CICGRU research leader. "Molecular markers are important tools for obtaining this information."

Sappington, David Grant, Nicholas Miller, and others at Ames are collaborating with university colleagues and the Institut National de Recherche Agronomique in France to conduct more sensitive analyses of genetic variation in populations. The scientists are taking specimens from WCR's native range in the Great Plains and trying to get better population-structure data than that obtained previously from the Corn Belt, where the pest appeared more than 40 years ago—not long when it comes to random genetic change in huge populations, says Sappington.

"By analyzing these long-established populations, we hope to estimate how far rootworm adults move per generation," he says. "Such estimates are important for accurately predicting the development and spread of *Bt*-resistance and rotation-resistance traits over long distances—almost impossible to do using conventional methods such as mark-release-recapture experiments."—By **Alfredo Flores,** formerly with ARS, and **Jan Suszkiw,** ARS.

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Adult female western corn rootworm, *Diabrotica virgifera virgifera,* on a corn leaf.