Gene Turns Fungus Against Itself

A clever killer lives in the cornfield, hiding in dried husks of dead plants. His weapon is a poison so deadly even he could succumb to its toxic powers without a means of self-protection. But someone got wise to the killer's game—and stole his secret to protect the innocent.

It sounds like a plot from film noir. But this detective story took place in a laboratory. The investigators were plant pathologists Greg Upchurch, with USDA's Agricultural Research Service, and Jon Duvick, with Pioneer Hi-Bred International, Incorporated.

The villain was a group of fungi in the genus *Cercospora*. These microbial miscreants secrete the toxin cercosporin, which allows them to feed on plant tissues. *Cercospora* fungi cause disease on many crop plants, including grey leaf spot on corn and purple seed stain on soybeans.

But growers are the real victims. Grey leaf spot can take 5 to 50 percent of a crop's yield. In 1996, roughly 80 percent of the cornfields in central and southern Illinois were hit. No-till cultivation, a must for saving soil from erosion, unfortunately gives this fungus more opportunity.

At the ARS Soybean and Nitrogen Fixation Unit in Raleigh, North Carolina, Upchurch and his research team isolated and cloned a gene that protects *Cercospora* fungi from their own toxin. The scientists determined the gene's function by inactivating it through mutation. In the presence of toxin, their mutant strain with the nonfunctioning gene had 60 percent less growth than a strain with an intact protective gene.

It appears the gene is responsible for making a protein that pumps the toxin out of fungal cells. Upchurch's research team named the protein "cercospora facilitator protein," or CFP. Could the fungi's secret weapon be turned into a plant protector, they wondered?

Finding the answer was critical. Although Upchurch's talented team discovered the unique *cfp* gene, getting it transferred into corn would take the skills of researchers at Pioneer.

"Many companies are looking for genes to put into plants for various reasons, and they have the skills and expertise to move these genes into crops," says Upchurch. "But finding specific genes can mean a lot of basic research, and this may not be an option for every company."

"We have our own large gene discovery group," says Pioneer's Duvick. "But when we hear about good research with real potential, we're always interested. No one company or research project will be able to discover all the genes."

The two scientists embarked on a cooperative research and development agreement that allows them to perform several joint research projects. Their goal: to determine whether the gene for cercosporin resistance can be used to protect other organisms. The agreement allows both partners to do more with their scientific resources.

"Information and materials being exchanged under this agreement give us another approach to our corn disease programs," says Roger Kemble, Pioneer's research director for crop protection. "We are pleased to work with USDA on this project."

To transfer a useful gene into crops, scientists must:

• First show that they can move the gene from organism to organism, a process called transformation;

• Be sure the new host organism adopts the new gene as part of its own genetic programing. In the case of the *cfp* gene, it means knowing that having the gene causes the new host organism to make the CFP protein;

• Prove that the new gene is effective—that it reaches plant leaves and wards off the toxin's effects; and

• Test the gene as part of the plant's total genetic package in the field.

ROB FLYNN (K8140-1)

Plant pathologist Greg Upchurch inspects tobacco plants inoculated with *Cercospora nicotianae* fungi for evidence of lesions indicating infection.

ROB FLYNN (K8138-1)

Soybeans with purple seed stain caused by *Cercospora kikuchii* rest on a printout showing the genetic code for cercospora facilitator protein.

Since corn is such a complex organism, a simpler one was sought for transformation and initial testing. Although brewers yeast is a favored organism for plant biotechnology, it seems to naturally resist cercosporin. Upchurch found that the cercosporin-vulnerable fungus *Cochliobolus heterostrophus* fit the bill.

C. heterostrophus transformed with the *cfp* gene did show resistance to cercosporin—a good omen for the gene's potential use in plants including new corn or soybean hybrids.

Achieving transformation and effective protection in *C. heterostrophus* gave Upchurch confidence to try putting the gene in tobacco plants, the classic "lab rat" of plant science. Pioneer Hi-Bred researchers began inserting the gene in corn.

Upchurch used a small piece of bacterial DNA called a plasmid vector. This plasmid vector served as a molecular "bus" to carry the *cfp* gene into plant cell nuclei. A culture of *Agrobacterium* containing *cfp* plasmid was used to infect the plant and transmit the *cfp* gene so that it could be incorporated into the plant's genetic programing. Although this technique can be used for many plants, it doesn't work well in corn.

To get the gene into corn, Pioneer brought a more recent innovation, a gene gun, to the investigative team. Corn can be more of a challenge to change genetically—that's why the gene gun was needed.

"There's probably no single reason why genetic transfer should be harder with plants like corn," said Duvick. "Someday, we'll probably find out that it's many unrelated things—that's how complex the plants are."

After the gene was transferred, Upchurch and Duvick needed to be sure their respective plants were actually making the protective protein. For this, Upchurch produced special antibodies to detect CFP in plants.

The antibodies are specific proteins that bind to the CFP protein much as human antibodies do with cold viruses. Producing antibodies in nature is a standard laboratory technique used in medicine and plant science. Upchurch's team got the antibodies by using fragments on the CFP protein that they suspected would trigger antibodies in an animal. These antibodies allowed them to confirm that the CFP protein was present in tobacco and corn.

But having CFP present is not enough. It has to be actively working in place in cells to protect the plant.

Pioneer plans to test CFP corn in a greenhouse and in test fields. Armed with this new genetic shield, it will soon meet *Cercospora* when scientists expose the corn to this fungal crop killer.

"This is only the first of many tests," says Duvick. "If it shows promise, we'll still have a long way to go. Good science isn't based on the evidence of a single summer's data."—By **Jill Lee,** ARS.

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When lesions caused by *Cercospora nicotianae* occur in large numbers, such as on this tobacco leaf, they cause significant leaf tissue death.