Researchers ID Gene For Plant Resistance

UNIVERSITY PARK (Centre Co.) — Researchers at Penn State and at Brookhaven National Laboratory have collaborated to identify a gene that is directly linked with a mechanism of plant resistance to insect pests.

The discovery, which was described in an article published in the Proceedings of the National Academy of Science on Aug. 6, could have important implications for agriculture as well as other industries.

David Schultz, who received his Ph.D. in genetics from Penn State's College of Agricultural Sciences in May of this year and is a postdoctoral researcher at Michigan State University, identified the gene in collaboration with June Medford, assistant professor of biology and biotechnology in Penn State's Eberly College of Science; Edgar Cahoon and John Shanklin, both biochemists at Brookhaven National Laboratory in Upton, N.Y.; and Richard Craig, J. Franklin Styer professor of horticultural botany, Diana Cox-Foster, associate professor of entomology and Ralph Mumma, distinguished professor of environmental quality, all in Penn State's College of Agricultural Sciences.

The isolation and identification of the gene is the culmination of years of interdisciplinary studies at Penn State, beginning with research looking at the susceptibility of garden geraniums to certain kinds of spider mites and aphids. Since the early 1960s, Penn State scientists have studied thousands of plants to learn what makes some geraniums resistant and others susceptible to pests.

A grant from the U.S. Department of Agriculture in the late 1980s and a four-year Penn State Intercollege Grant beginning in 1990 supported the highly multidisciplinary research effort, which included horticulturists, plant morphologists, entomologists, geneticists, molecular biologists, and biochemists.

The researchers determined that resistant geraniums produce a viscous material from hairlike structures, called trichomes, on their stems and leaves. This material impedes insects and mites in several ways — by trapping them in the viscous liquid, by killing them outright, and by inhibiting the ability of females to lay eggs. The viscous liquid contains compounds known as unsaturated anacardic acids, which are synthesized from unsaturated fatty acids in plants.

"Trichomes in both resistant and susceptible plants produce anacardic acids, but the difference is that anacardic acids in resistant plants are unsaturated," said Schultz, who did his research in June Medford's laboratory. "Unsaturated anacardic acids form a viscous liquid, like vegetable oil — so they stick to the insects. You can feel these sticky substances on the stems of the resistant geraniums. The saturated anacardic acids found on susceptible plants are more solid, and do not stick to insects."

Before Schultz began his doctoral studies at Penn State in 1992, a group of students and postdoctoral scholars working with Craig and Mumma had identified the two unsaturated fatty acids that were the precursors to the unsaturated anacardic acids in the resistant geraniums. They also knew that a single gene was responsible for the formation of the unsaturated fatty acids. "My role was to find that gene," Schultz said.

Because the researchers knew that the sticky compounds could be found only in the trichomes, they needed a method to obtain large quantities of them. Postdoctoral scholar Ellen Yerger, working in Diana Cox-Foster's laboratory, developed a method of removing the tiny trichomes from the plants so that their genetic makeup could be examined. By freezing the parts of the plant covered with trichomes in liquid nitrogen and then vigorously shaking them, she could get the trichomes to break away from the supporting plant tissue.

"This was crucial for my research because the gene I was looking for could only be found in these trichomes," Schultz said. "It took months to collect enough trichomes so that I could begin my research. Without Ellen's technique, my work would have been impossible."

The type of gene Schultz was looking for encodes a desaturase. "It desaturates the fatty acid, changing its molecular structure by adding a double bond at a specific point along the fatty acid's carbon chain," Schultz said. "I was looking for the desaturase gene that leads to the production of two fatty acids known as $16{:}1\Delta^{11}$ and $18{:}1\Delta^{13}$.

"What we were hunting for was a gene that looked like it had the characteristics to convert fatty acids in precisely the way necessary to create the unsaturated anacardic acids," Schultz said. "We also knew from previous research that this gene would be expressed only in the trichomes of the resistant plants and nowhere else — not in other parts of the plants, and not in the trichomes of the susceptible plants."

Next, Schultz and Medford did RNA assays, which analyzed the expression of genes in the trichomes of resistant geraniums, in those of susceptible geraniums, and in tissue from other parts of the plants. They narrowed the search to two genes that they thought might encode the desaturase responsible for the production of unsaturated fatty acids. "One of the genes was a perfect match," said Medford. "We found it only in the trichomes of the resistant plants, and nowhere else. The gene was present in all resistant plants regardless of whether they were parents, hybrids or progeny from our genetic experiments. We were pretty sure this was our gene."

But the researchers needed more evidence before they could say that this definitely was the desaturase gene they were looking for. "We next had to follow up with a biochemical evaluation," said Schultz. "That is, we needed to insert the gene into some type of living tissue and see if it actually would convert the saturated fatty acids to unsaturated fatty acids, leading to the production of the sticky material in the resistant geraniums."

The researchers had planned to insert the gene into plant tissue and look for the formation of these fatty acids, a time-consuming study involving growing the plants to maturity. But in December 1994, at a conference in Annapolis, Md., Medford met biochemist John Shanklin, who worked at Brookhaven National Laboratory. "This was extremely lucky for us, because it just so happened that he was working with a method of desaturase gene expression using the bacterium E. coli," Medford said.

"Because E. coli very quickly expresses genes that are inserted into it, using this method can considerably speed up some genetic research," said plant biochemist Edgar Cahoon, who works in Shanklin's lab. "Our research fit perfectly with the study being conducted at Penn State."

Teaming up with Shanklin and Cahoon enabled the Penn State researchers to insert the gene into E. coli, allowing it to be expressed in the bacterium and resulting in the production of two unsaturated fatty acids new to the E. coli. The researchers then isolated enzymes from the E. coli and placed them in contact with various saturated fatty acid substrates. The interaction of the enzymes with the proper substrate showed whether the gene encoded a functional desaturase and identified the preferred substrate.

"We expected the desaturase encoded by our gene to act on the saturated fatty acids known as palmitic (16:0) acid and stearic (18:0) acid," said Schultz. "We discovered that the pathway actually was more complex. Instead of desaturating these two fatty acids, the gene encoded a protein that acted on a different fatty acid, known as myristic acid (14:0) and converted it to one called myristoleic acid ($14{:}1\Delta'$). This fatty acid was then converted into the $16{:}1\ \Delta^{11}$ and $18{:}1\ \Delta^{13}$ fatty acids by the elongation of $14{:}1\Delta'$. The behavior of the desaturase was somewhat different from what we expected, but the outcome showed that we definitely had identified the desaturase gene associated with plant resistance. I see this as the first step in defining one pathway of pest resistance in plants at the molecular level."

Penn State is currently in the process of patenting the use of this gene, which could have applications for both agriculture and industry. When more genes that play a role in this mechanism of plant resistance are identified, plant geneticists may be able to transfer the mechanism to important crops such as tomatoes and potatoes. "These plants are members of the Solanaceae family, and have similar trichomes," said Medford. "Making tomatoes and potatoes more resistant to certain pests would be extremely helpful for growers. In addition, understanding the mechanism of pest resistance will greatly benefit plant breeding and integrated pest management programs."

Another application could benefit both agriculture and industries that require specialized oils. "These oils are expensive to manufacture, but they might be produced far more cheaply in plants or in microorganisms," said Medford. "This could help farmers, who could grow crops that produce these specialty oils."

"The faculty at Penn State did a truly admirable job over the years of combining individuals with complementary talents and abilities in this research program," said Andrew Benon, professor emeritus of marine biology at Scripps Institute of Oceanography, University of California at San Diego. "The result is that Schultz and others have built on the past research and have made a briliant discovery."

"This is research at its best — it makes profound contributions to both basic and applied science," said Stuart Patton, Evan Pugh professor emeritus of agriculture at Penn State, who informally reviewed the researchers' paper. "This team was highly ingenious in relating genetics and lipid biochemistry to insecticidal properties of unique fatty acids in geraniums."

"One individual could not have accomplished this all alone," said Schultz. "No one scientist could have all the skills or knowledge necessary — not to mention there are decades of research leading up to the discovery. It was truly a collaborative effort, and being part of it was a great learning experience."

Changes In Store For Angus Herd

COLLEGE PARK, Md. — Dr. Thomas A. Fretz, dean of the College of Agriculture and Natural Resources at the University of Maryland at College Park, has announced that major changes will be made to the Wye Angus beef herd beginning Sept. 1. The herd is located at the college's Wye Research and Education Center near Queenstown.

The beef herd changes are designed to ensure full utilization of the herd for the improvement of Maryland's beef industry. They will be overseen by Dr. Scott Barao, associate professor of animal sciences, who will assume programmatic responsibility for the herd.

During the next year:

- The size of the base herd will be reduced to a level better matched to the college's available resources. The cow herd will be reconfigured to more closely reflect the unique germplasm base developed by Jim Lingle and gifted to the university by Arthur Houghton.
- The 1997 Wye Sale will include a larger than usual offering of select Wye-based females. Future sales will occur as needed based on the availability of excess females or superior genetics that will benefit Maryland's cattle industry.
- An open Maryland Bull Test Station will be established at the Wye REC in 1997. Details concerning the test will be made available this winter.
- An industry advisory committee will be formed to ensure close communication and collaboration between the Maryland beef cattle industry and the university.

The beef cattle research program conducted at the college's Wye Research and Education Center will focus on addressing both current and future needs of the industry.

"We have a unique opportunity to pursue important beef research using a forage-based cow/calf production system with a highly defined genetic base," said Barao. "We will build our efforts around the concept of integrated resource management (IRM), and we anticipate creating a beef research, extension and teaching program that will be highly productive and responsive to the needs of our citizens."

Eddie Draper, along with Dr. Russell Brinsfield, center head of the Wye REC, will assist Barao in these efforts by assuming day-to-day responsibility of the Wye herd. Draper has worked with the herd for 10 years and is looking forward to being a part of a viable and active research program