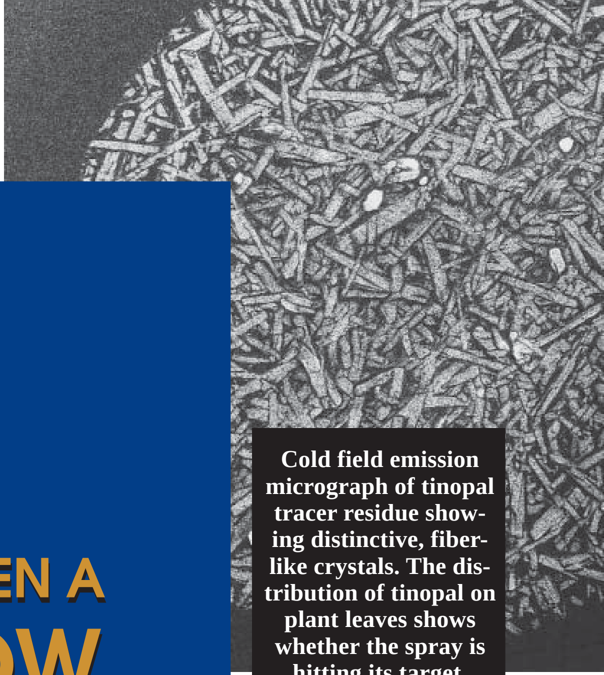
Using Space-Age Technology To Open A New Window Into The World of Horticulture



Cold field emission micrograph of tinopal tracer residue showing distinctive, fiber-like crystals. The distribution of tinopal on plant leaves shows whether the spray is hitting its target. Magnified about 800x. Photo by Charles Krause. (K9209-3)

You'll have a better chance of getting a beautiful and disease-free poinsettia, mum, or begonia in a few years as scientists get a fresh view of the world of horticultural pests.

Armed with space-age technology similar to that used to photograph the surface of Mars, agricultural scientists are peering into the surface topography of nursery crop leaves to see what pesticides and biological control organisms work best, says plant pathologist Charles R. Krause. He's with the ARS' Application Technology Research Unit, in Wooster, Ohio.

But instead of a NASA mission control center, this work is emanating from the newly created Molecular and Cellular Imaging Center in Wooster. The center—a joint venture between ARS and Ohio State University (OSU)—has four of the newest generations of microscope: two types of scanning electron microscope, a transmission electron microscope, and a confocal laser scanning microscope that produces three-dimensional fluorescent images. The microscopes have digital imaging features originally developed for telescopes and cameras in the space program to study the surface of planets and their moons.

The center—formerly called the Electron Microscopy Laboratory—also houses a DNA high-throughput sequencer, so scientists can perform genetic and molecular studies.

"The lab is shared equally by OSU and ARS scientists in all disciplines," says Steven A. Slack, director of OSU's Ohio Agricultural Research and Development Center, in Wooster. "We couldn't have outfitted it so well if it weren't for our close partnership with ARS."

Exploring the Leaf Surface

These advanced microscopes and related equipment allow Krause and colleagues to peer closely into the ecology of the leaf surfaces of plants, a world inhabited by fungi and bacteria—good and bad. Krause views fungicides, bactericides, insecticides, and herbicides flowing through the waxy peaks and valleys of the leaf surface, just as water flows on planet Earth. The scientists are tracking pesticides to see whether they reach the underside of plant leaves, where diseases and other pests tend to begin their attack. The plant samples come from the many Ohio commercial nurseries and greenhouses that Krause and colleagues work closely with.

"The capabilities offered by the new center will help us better assess how well a particular pesticide is reaching the pest, how uniform the pesticide application is, and how well the sprayers and other applicators are working," says Ross D. Brazee, who heads ARS' Application Technology Research Unit.

Krause agrees: "We'll be seeing things we could only infer before." He's already used the microscopes to see the spores of the fungus that causes apple scab disease as they emerge from crabapple leaf surfaces. And next to the spores he has seen crystals of a fungicide that controls those spores. Once the spores emerge, they spread by being blown in the wind; washed off by rain or irrigation; or brushed off by insects, animals, or people.

Krause has also seen gray mold disease up close. Caused by *Botryis cinerea* L., it's a common disease that is costly to greenhouse producers. Infecting fruits, flowers, and trees, gray mold looks like gray fuzz and is found on aging blossoms and soft ripe fruits. It first attacks dead or dying plant parts, then moves to the healthy parts. When infected flowers or leaves are plucked, a grayish-white cloud of fungal spores can usually be seen.

These are just two of the many fungal or bacterial pests that rob a fair share of the nursery and floriculture industry's returns of $12 billion a year—one of the fastest growing areas of U.S. agriculture. They are also the pests that can brown up a golf green and destroy precious landscape plants, not to mention corn, soybeans, wheat, and other major food crops.

A Million Times Life-Size

The new scanning electron microscopes and related equipment offer the scientists an opportunity for much greater magnification—up to 450,000 times, compared to 75,000 times for conventional scanning electron microscopes. The transmission electron microscope can magnify to over 1 million times. And the resolution, or clarity, of the images from all the new microscopes is much better.

"For example, the resolution of one of the scanning electron microscopes, called a cold field emission microscope, is five times better than the conventional scanning electron microscopes of the past," Krause says. "We can see things clearly down to 15 angstroms instead of 70 angstroms. An angstrom is only the diameter of a hydrogen atom, and many of the structures we will be looking at—such as viruses and cellular organelles—are 100 to 200 angstroms in size."

A Really Cool Microscope

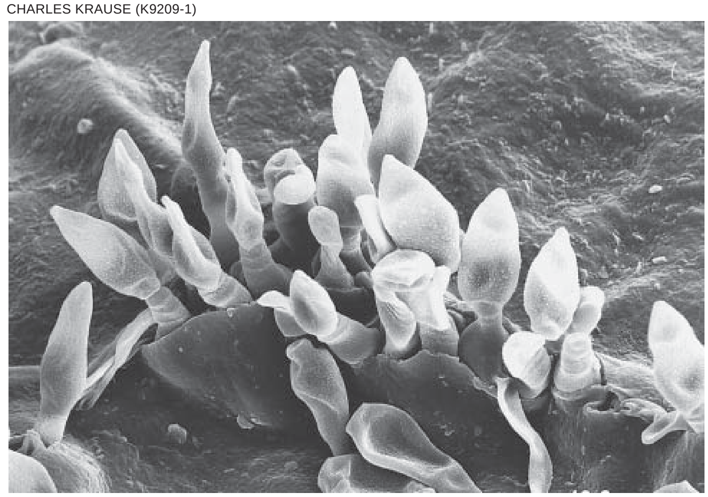
The cold field emission microscope eliminates the conventional electron source that illuminates the sample. That source generated so much heat—up to 2,700˚F—that it gave scientists very little time to observe a fresh sample before it cooked beyond recognition. The hot electron source has been used on most scanning electron microscopes since their introduction in 1965. But the cool temperature microscope lets scientists observe the fungicides and organisms as they appear in real life and in real time.

The cold field emission microscope is connected to an x-ray analyzer that determines the chemical and physical structures of the plants, pests, biocontrol organisms, and pesticides. "Not only can we view the fungicide coverage, but we can also

PEGGY GREB (K9164-1)

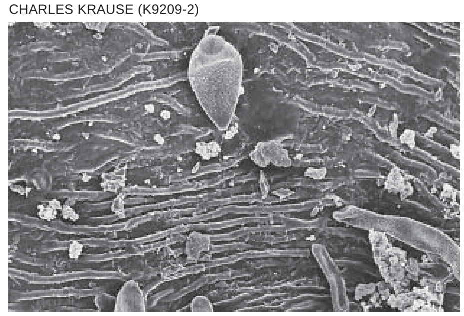
ARS plant pathologist Charles Krause uses a cold field emission scanning electron microscope to study pesticide distribution on plant leaves.

CHARLES KRAUSE (K9209-1)



Fungal spores that cause apple scab disease erupting through the cuticle of a crabapple leaf. Magnified about 1500x.

CHARLES KRAUSE (K9209-2)



Fungicide crystals (arrows) next to spores of the apple scab fungus, *Venturia inaequalis.* Magnified about 800x.

PEGGY GREB (K9161-1)

Left to right: Ross Brazee, head of the ARS Application Technology Research Unit, floricultural producer Justin Marotta, and agricultural engineer Richard Derksen discuss fungicide distribution.

PEGGY GREB (K9162-1)

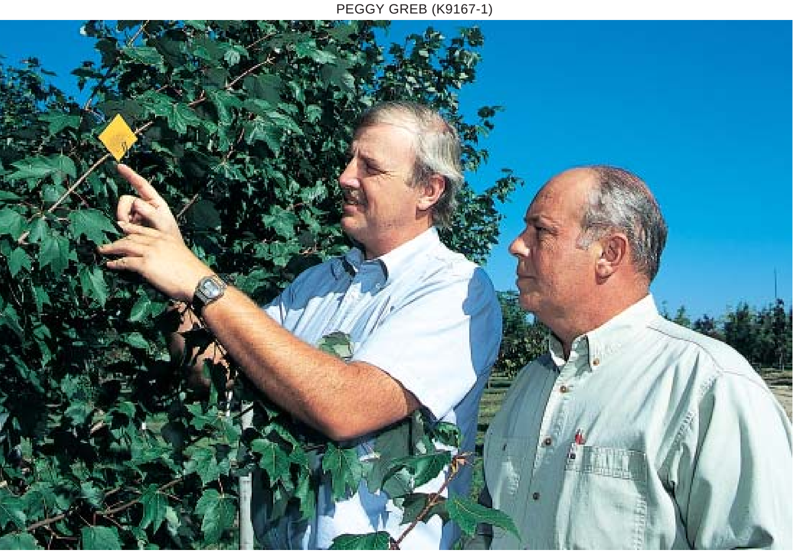
ARS technician Leona Horst loads a fuchsia leaf sample into the variable-pressure scanning electron microscope.

identify the chemicals in the pesticide residue," Krause says. This ability allows the team to distinguish fungicide crystals from extraneous dust and dirt contaminants they can resemble. "We use all of this equipment to answer the industry's questions about the effectiveness of new fungicides—both chemical and biological—and the performance of new spray equipment," he says. He's named the use of such microscopes to analyze plant diseases and pesticides "electron beam analysis."

The scientists' explorations should help make it more profitable for the nursery and greenhouse industries to produce the plants that beautify our homes and neighborhoods.

Justin Marotta expects that to be the case for the hundreds of fuchsia varieties he raises at his Bellville, Ohio, greenhouses.

PEGGY GREB (K9167-1)



Ohio State University extension agent Randy Zondag (left) shows nurseryman Robert Lyons coverage of tinopal tracer on a water-sensitive card.

He helped the scientists in a study comparing an electrostatic sprayer with a cold-fog sprayer. An electrostatic sprayer has a nozzle that charges tiny pesticide droplets with static electricity so they cling to plant leaves. Cold-fog sprayers apply pesticide in a mist or aerosol.

After Marotta's nursery workers sprayed a copper fungicide, the scientists looked at leaf samples under the cold field emission microscope to study spray penetration of the leaf canopy. They found that the electrostatic sprayer consistently provided more uniform plant coverage than the cold fogger.

"It will take many more experiments to see if this finding holds even for the conditions in the study, let alone for other situations," says Krause. "But this first test of the equipment showed us that it performs well with fresh samples and will allow us to observe plant-parasite-pesticide interactions live."

The other new scanning electron microscope has a variable pressure feature that allows scientists to screen plant samples at low magnification and at low pressure, avoiding the tissue damage that high pressure can inflict. It serves as an intermediary between the old and new microscopes, Krause says.

The third new instrument is called a transmission electron microscope. It transmits electrons through ultra-thin tissue sections for an internal look at the fine structure of cells, rather than scanning the cell surfaces.

A 3-D Glimpse Into Plant Space

The fourth microscope, an earlier acquisition, is called a confocal laser scanning microscope. It scans optical "slices" of the specimen and reassembles them by computer into three-dimensional images of the cell structures of plants or animals. Used with a fluorescent dye, it can show a plant's response to disease.

"Since the sample isn't literally being sliced, we can see cross-sectional views of living cells, tissues, and organisms

PEGGY GREB (K9166-1)

David Geisler of Possum Run Greenhouse in Bellville, Ohio, uses an electrostatic sprayer to apply a fungicide to poinsettia plants. SEM technology showed that this type of sprayer covered plants more evenly than cold fogger sprayers.

without harm. That means we can observe the invasion of plant tissue by disease bacteria or viruses over time," Krause says. "There are very few such microscopes at agricultural colleges or universities."

Krause plans to evaluate sprayers and fungicides used by nurseries to fight scab on apple and crabapple trees. He also plans experiments to search for safer fungicides like calcium chloride, the same material that is used as de-icing salt, to fight gray mold disease.

Krause also plans tests of the spray coverage of foggers and smoke bombs used in greenhouses to distribute pesticides in the air. "This will allow us to know exactly how much residue the spray leaves and when that residue dries, so the industry and the U.S. Environmental Protection Agency will know how long workers need to stay out of the greenhouses after pesticide applications," Krause says.

"We want to help the industry in its commitment to lower pesticide use where possible and to use the safest pesticides possible, with the safest techniques possible," Krause says.

Robots To Spray Pesticides at Night

The American Nursery and Landscape Association (ANLA), in Washington, D.C., sees this new research as very promising. As part of a national Floriculture and Nursery Research Initiative, the industry, along with USDA, Ohio State University, and other land-grant universities, is coordinating robotics research at Carnegie-Mellon University in Pittsburgh, Pennsylvania, and the pesticide application technology research at Wooster, Ohio.

"Imagine robotic pesticide application done in greenhouses and nursery fields at night, when all the workers have gone home. It would give a 12-hour jump on any safe re-entry time limits set by EPA, and the pesticides wouldn't be as toxic to plants because the temperatures would be cooler. And winds are less, so drifting is minimized," says Ashby Pamplin, ANLA's director of horticultural research. "Combine the robotics with the sophisticated analyses Krause and his colleagues are using to perfect pesticide applicators, and you have a very promising scenario for the industry.

"Krause and his unit are models for federal scientists who work with industry—they really listen to what we have to say. The initiative has created a team approach that coordinates the research of many scientists in many locations to synergize their work. Only ARS is in a position, with its nationwide network of labs, to make this happen," says Pamplin. The initiative's proposed initial annual ARS funding increase of $20.7 million includes $4.8 million for postharvest research. Some of this money will go to the Carnegie-Mellon and Wooster researchers.

"These partnerships go way beyond finances or a physical building," says Slack. "It's the spirit of working together for a common cause. We complement each other's research as we work jointly with the same goals in mind. It's a great example of how a partnership should work, in every sense of the word. It helps each organization meet—and exceed—its goals."—**Don Comis,** ARS.

This research is part of Plant Safety (#303) and Crop Protection and Quarantine (#304), two ARS National Programs described on the World Wide Web at http://www.nps.ars.usda.gov.

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