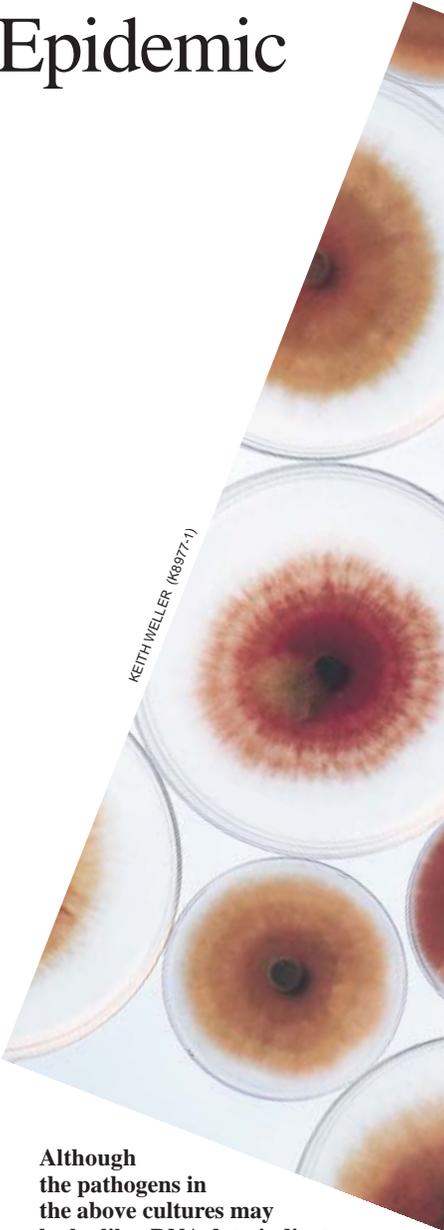


## Guarding Against a Plant Disease Epidemic



KEITH WELLER (K8977-1)

Although the pathogens in the above cultures may look alike, DNA data indicate they represent distinctly different species within the *Fusarium graminearum* complex.

Curiosity compels some people to research their family tree. But to ARS scientists like microbiologist Kerry O'Donnell and molecular geneticist Corby Kistler, reconstructing the genealogy of plant pathogenic fungi goes beyond curiosity.

O'Donnell, at Peoria, Illinois, and Kistler, at St. Paul, Minnesota, along with colleagues at North Dakota State University (NDSU), Fargo, are doing research that may help keep grains from becoming contaminated with toxins that threaten food safety and plant health.

Kistler and O'Donnell are among scientists researching toxin-producing *Fusarium* fungi. These fungi are notorious for causing a disease called scab, or Fusarium head blight, in grains such as wheat and barley, as well as ear and stalk rot of corn. One of their goals is to develop technology for identifying which species cause disease. They also hope to determine each species' range of hosts, geographic distribution, and potential for producing mycotoxins. This information is urgently needed by plant breeders and disease control specialists worldwide to develop effective control measures to minimize the threat of scab.

### One Disease—But Eight Causes!

At the National Center for Agricultural Utilization Research (NCAUR) in Peoria, O'Donnell and molecular geneticist Todd J. Ward are using DNA evidence to measure the genetic diversity of pathogenic fusaria that infect plant hosts throughout the world.

In the past, all scab epidemics worldwide were thought to be caused by a single pathogen. But new evidence proves that's not true. "To our surprise and dismay, our DNA data so far reveal at least eight genetically distinct scab pathogens, all of which are being called *Fusarium graminearum*," says O'Donnell. "We recognize them as distinct species based on genealogical evidence obtained from six different genes."

Kistler, who works at the ARS Cereal Disease Laboratory in St. Paul, found that all eight pathogens caused the devastating scab disease on wheat in greenhouse experiments. Six of the eight species are known to infect corn.

In the Department of Veterinary and Microbiological Sciences at NDSU, mycotoxin chemists Howard H. Casper and Beth K. Tacke found that all eight scab-causing *Fusarium* species produce similar toxins on grain. They also found that the scab species could not be distinguished from one another by the toxins they produced.

"We're confident that our multidisciplinary genetic research, coupled

KEITH WELLER (K8976-1)

Geneticist Corby Kistler looks for differences in wheat disease symptoms caused by divergent strains of *Fusarium* to determine how the fungus spreads within the plant.



with the toxin and pathogenicity data, will help plant breeders develop varieties with broad-based resistance to *Fusarium* and thereby avert scab epidemics,” says Kistler. According to O’Donnell, epidemics may also be averted if the research alerts USDA’s Animal and Plant Health Inspection Service officials to potential sources of rogue fungi secretly inhabiting grain and other plant materials exported from one country to another.

### Global Significance

Most wheat and barley varieties currently produced are susceptible to scab. Because of yield losses and price

discounts, the disease costs U.S. wheat farmers at least \$2.6 billion in the 1990s. Add to that losses from recent outbreaks in Asia, Canada, Europe, and South America, notes Kistler, and it’s obvious that scab is truly a global problem. The disease has emerged as one of the most important plant diseases worldwide within the past decade.

When wheat is infected, the grain typically becomes discolored and shriveled. Often it’s unsuitable for food or livestock feed because it may be contaminated with trichothecenes and other chemicals that overstimulate estrogen production in animals. Human exposure to these toxins results in a variety of symptoms

including acute dermatitis, diarrhea, and hemorrhaging.

Although several species of *Fusarium* have been isolated from scabby grain in the Midwest, Kistler says the primary scab pathogen in the United States is the “real” *F. graminearum*, not to be confused with its closely related, look-alike “cousin” species that are part of the *F. graminearum* family tree.

With the advent of molecular genetics tools, the DNA data gathered by O’Donnell and Kistler show that only one of the eight species within the *F. graminearum* family tree is responsible for recent scab outbreaks and epidemics within the United States.

Based on similarities and differences in DNA sequence data, O’Donnell has deduced the species’ evolutionary histories and depicted their relatedness in a genealogical chart called a phylogenetic tree. Ward recently joined O’Donnell’s laboratory to gain insights on how the scab pathogens developed their ability to cause plant disease. In practical terms, that means he wants to use molecular genetic data to more easily pinpoint the key genes contributing to the pathogenicity of the various species. Another goal is to use information about the global distribution of *Fusarium* species to gain insights into their ability to cause disease on distantly related plants of economic importance.

In Kistler’s laboratory, postdoctoral geneticist Liane Rosewich has begun research on the potential emergence and spread of new pathogenic strains—an area of research known as population genetics. By observing DNA patterns from scab-causing fungi collected throughout the Midwest, the scientists can determine the presence of one or more genetically distinct pathogens and whether hybridization may be occurring in farmers’ fields.

Then, using satellite-based global positioning systems to mark the precise spot where samples were taken, researchers can monitor the spread of pathogenic





*Fusarium graminearum* is responsible for most of the wheat scab and corn ear rot in the United States. Above, geneticist Liane Rosewich examines one of the many cultures of this species collected by the ARS Cereal Disease Laboratory.

strains from season to season to predict the outcome of disease spread in the field. But more than that, their goal is to find ways to most effectively manipulate cultural practices to shift the balance of the plant and fungus interaction in favor of plant health.

### DNA Database

Until now, most scientists throughout the world have had to use visual means to identify *Fusarium* pathogens. But as curator of *Fusarium* in the ARS Culture Collection housed at NCAUR, O'Donnell and his colleagues Ward and technician Eileen Sullivan are developing a comprehensive DNA database for all toxin-producing and disease-causing *Fusarium* species.

By comparing each pathogen's DNA with DNA collected from more than 3,000 strains, the team has discovered more than 100 new *Fusarium* species within the ARS collection in the past 5 years, including the 8 researched at St. Paul and Fargo. The ARS Culture Collection includes the most complete,

genetically characterized collection of fusaria in the world.

While developing molecular markers for fusaria, O'Donnell uncovered clues that threats of scab could be lurking in some unlikely places. Although the name *graminearum* means "of the grasses," O'Donnell says several of the species are known to occur only on trees, shrubs, or vines in Africa and South and Central America.

O'Donnell analyzed the DNA of one species within the *F. graminearum* family tree that was isolated from diseased tissue of a tropical ornamental called grape ivy or oakleaf ivy (*Cissus rhombifolia*). The infected plant was believed to have been imported into a garden center in Pennsylvania from South or Central America, where it is known as Venezuelan tree-bine. Fortunately, the *Fusarium* inhabiting this common horticultural houseplant is not known to cause scab in U.S. grain. But this fungus is among the eight that Kistler found pathogenic to wheat and that Casper and Tacke found also produced toxins.



Microbiologist Kerry O'Donnell stores genetically characterized wheat scab pathogens in liquid nitrogen for future studies.

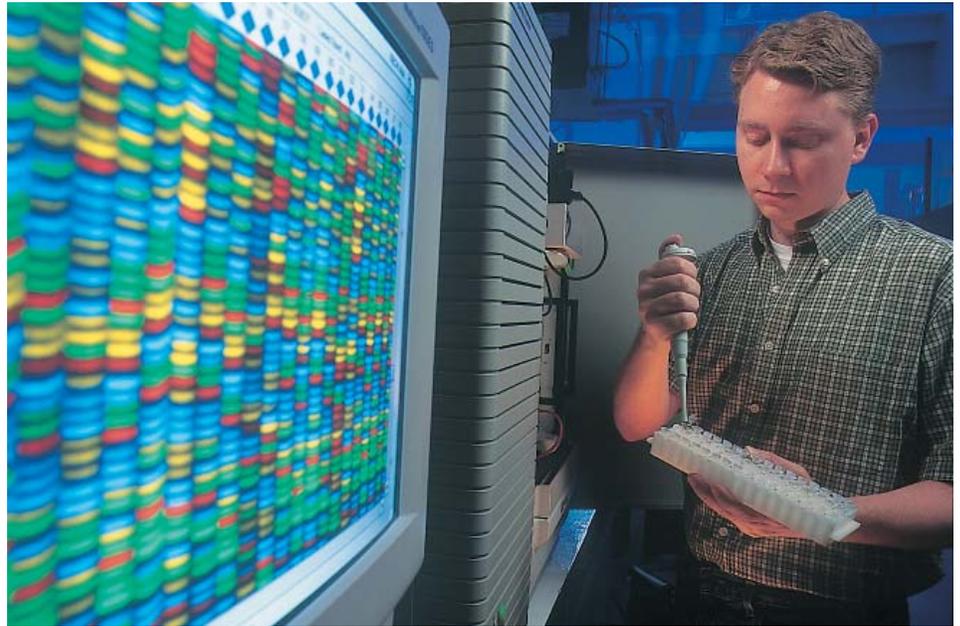
### New Threats From Hybridization and Evolution

O'Donnell and Kistler made a disturbing discovery when they examined strains isolated from moldy corn in Nepal. One strain had a mixture of DNA from two *F. graminearum* family tree species thought to be geographically isolated—one from Africa and one from Asia.

"We theorize that the African species was recently introduced to Asia on moldy corn exported from Africa," says O'Donnell.

The findings graphically illustrate that international horticultural and agricultural trade in plants and plant products can bring together closely related species that have evolved in isolation from one another.

Could previously isolated species hybridize and become a serious plant disease threat? Natural hybridization of two fungal species, also called interspecific hybridization, may be rare, O'Donnell says, but such incidences seem more likely when a fungus is introduced into



**In research to control the spread of cereal pathogens, geneticist Todd Ward uses DNA sequence data to track the global distribution of wheat scab.**

a new habitat occupied by a closely related species.

One major lesson: Scientists and government officials involved with quarantine regulations, plant disease prevention and control, and plant breeding need to appreciate the tremendous genetic diversity and global distribution of *Fusarium* species that might cause scab, says O'Donnell. For plant breeding programs to be fully effective, for example, breeders need to take into account all eight *Fusarium* species.

### Deducing Geographics

Ward, O'Donnell, and Sullivan have recently developed a DNA-based diagnostic procedure that can be used to rapidly and accurately distinguish the eight species within the *F. graminearum* complex. From the data, they can reasonably deduce the pathogen's geographic origin. By learning which plants from various parts of the world may be most likely to harbor the pathogens, quarantine officials should become better able to prevent new scab epidemics within the United States.

Of the eight scab pathogen species, the researchers found that three appear to be native to Africa, two to South and Central America, one to New Zealand, and one to Asia. "The other species—like the common cold—is widespread," says O'Donnell, "although it is most common in the Northern Hemisphere."

Just as patterns of DNA sequences in humans suggest to researchers that human origins are in Africa, O'Donnell and Kistler's DNA evidence implies a similar genesis of the scab pathogens. Their data suggest that the pathogens appear to have initially evolved in Africa and South America and then spread into the Northern Hemisphere millions of years before the origin of agriculture, which dates back only an estimated 10,000 years. Insofar as agriculture and world trade developed relatively recently, the scientists believe the scab pathogens evolved in an environment of geographic and host isolation.

Now that Kistler realizes that the eight scab species can infect wheat and knows that at least six can infect corn, the scientists are concerned about the possi-

bility of new pathogens evolving through hybridization.—By **Ben Hardin**, ARS.

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