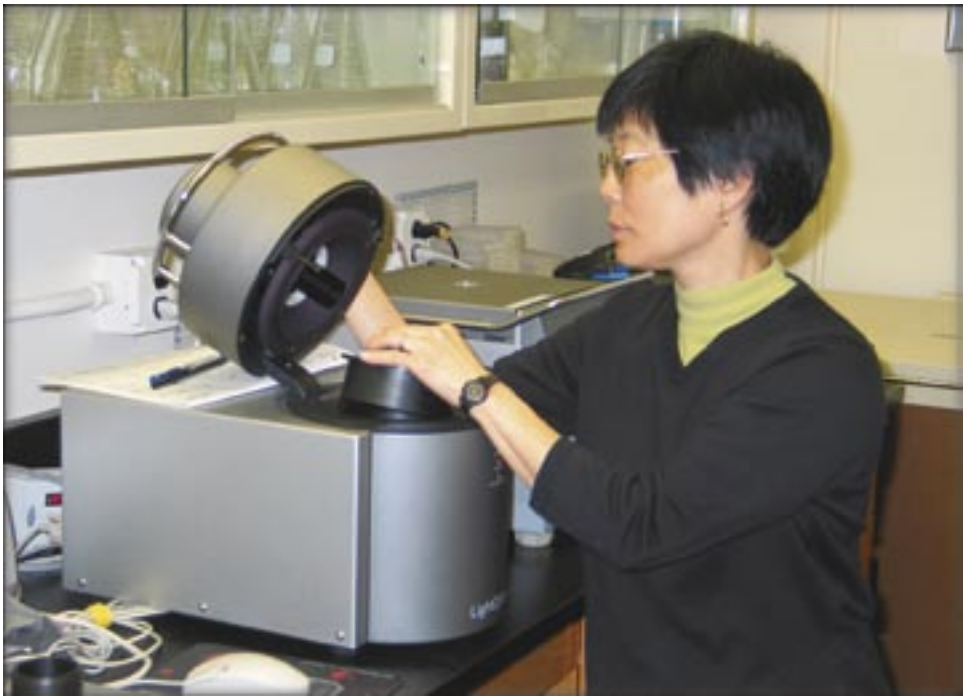
Okubara and Paulitz cracking the code for root disease detection

BY ERIC ZAKARISON

Improved control of cereal root diseases is an elusive goal for wheat growers and scientists. *Rhizoctonia, Pythium,* Take-all and *Fusarium* fungal diseases continue to exact a toll on Pacific Northwest (PNW) wheat yields despite efforts to limit their impact with rotations and cultural practices. Improved seed treatment technology provides some limited protection against these soil outlaws; however, efforts to develop new fungicides, bio-control agents and wheat varieties with disease tolerance are confounded by the complexity of pathogen identification. Due to the hard work of some dedicated, USDA-Agricultural Research Service (ARS) scientists, that's all changing.

Patricia "Pat" Okubara and Tim Paulitz are part of the USDA-ARS Root Disease and Biological Control Laboratory team, based at the Washington State University (WSU) campus in Pullman. Okubara is a plant molecular biologist, focusing on host-microbe interactions, and specifically on wheat's genetic response to root diseases. Paulitz, who was featured in a December 2002 *Wheat Life* article, works closely with Okubara and researches identification, ecology and epidemiology of root disease pathogens. Along with team leader, David Weller, and colleague, Linda Thomashow, they are at the forefront of cereals root disease research.

Okubara and Paulitz are currently developing a rapid and sensitive test to detect pathogens in cereal root and soil samples. The Washington Wheat Commission is proud to fund this pioneering research.



Pat Okubara, USDA-ARS plant molecular biologist, working with the LightCycler Quantitative PCR machine for DNA analysis, in her laboratory on the WSU Pullman campus.

Rapid tests for *Rhizoctonia* and *Pythium*

Plant pathologists trying to identify root pathogens in soil samples from wheat fields have previously sent them to a private, Australian laboratory for testing. Okubara, Paulitz and Ph.D. graduate student Kurtis Schroeder are developing rapid and sensitive tests to diagnose *Rhizoctonia* and *Pythium* in root tissue and soil samples for PNW wheat producers. More importantly, these tests, or assays, are being developed for root disease pathogens endemic to this region. Often the Australian tests are not tailored for the various species of *Pythium* and *Rhizoctonia* found in the PNW, which is crucial when screening wheat germplasm for disease tolerance or advising growers about cultural controls. What's more, these rapid tests will take only a day or two to complete, allowing growers or wheat breeders to receive results quickly. The Australian test results, on the other hand, typically take 30 to 60 days because of shipping time, which is inconvenient for making management or plant breeding decisions.

Probing the code

The key to developing rapid tests for identification of fungal root diseases is mapping unique parts of the pathogen's genetic code, then creating genetic probes, called primers, which target these unique sequences. Okubara, Schroeder and Paulitz create these primers, which are capable of pairing with nucleic acids on the pathogen DNA strands. The primers are manufactured in pairs because DNA has two strands, and the primers

Root Disease Management Strategies

- spread chaff and straw at harvest
- eliminate green bridge between crops with non-selective herbicide
- treat seed with protectant chemicals
- disrupt soil in seed zone when planting
- proper placement of fertilizer below seed
- root diseases may decline over time in long-term no-till
- new tests for root diseases will facilitate breeding and screening for disease tolerance

Tim Paulitz and Pat Okubara working with the ABI 3100-Avant Genetic Analyzer.

must pair with a target site on each of the strands to be functional.

Once primers for *Rhizoctonia* and *Pythium* are created, they are tested for their utility in identifying only the target pathogen and not other pathogen species, or various soil-borne fungi that might be present. The researchers replicate or "amplify" pathogen DNA using the new primers and a polymerase chain reaction (PCR) machine, which facilitates pathogen detection. The PCR machine is programmed to amplify only DNA from specific root disease pathogens that cause significant crop damage—no small feat considering the potpourri of DNA these scientists must sort to isolate the root pathogen.

Pythium primers

To date, Okubara, Schroeder and Paulitz have developed 30 pairs of DNA primers for *Pythium* detection, and are now tackling *Rhizoctonia oryzae* and *Rhizoctonia solani*, which proved to be more complex than first anticipated. As more primers are created and soil/tissue testing techniques are perfected, the team will use data collected at various eastern Washington sites (e.g. WSU Dryland Research Station at Lind, Cunningham Agronomy Farm near Pullman and WSU Variety Testing sites) to establish soil and tissue baseline levels for root disease. By knowing what "normal" soil or plant tissue populations of *Pythium* or *Rhizoctonia* species should be, they will be able to compare laboratory test results of soil and tissue samples submitted by growers to known levels of infection measured at test sites. This will also allow scientists to partially predict disease impact on crop yield.

Okubara's and Paulitz's research goals:

- Rapidly identify root disease pathogens;
- Quantify pathogens in soil or plant tissue; and
- Provide information about pathogen populations to growers and scientists. This information will allow growers to adjust cultural practices or rotations as needed for disease control, and aid wheat breeders in screening germplasm for disease tolerance.

Laboratory testing for root disease is still dependent on monitoring pathogen populations in fields across the growing region.

"*Pythium,* soil and climate interact in the environment," said Paulitz. "Tolerance may exist (in small grain varieties), but break down under certain conditions."

For this reason, laboratory and greenhouse screening of wheat germplasm for root diseases, while valuable, will never entirely replace variety field trials for testing disease tolerance. However, the rapid pathogen tests will be valuable tools to assess tolerance in both greenhouse and field trials.

Okubara said their research might also lead to more fundamental knowledge of wheat-disease interactions.

"We need to learn more about wheat's tolerance to root diseases, and its defense response," said Okubara. "Is there anything in wheat that could interact with a bio-control agent, for example, to enhance disease tolerance?"

Okubara said that anti-fungal compounds might also influence wheat's pathogen resistance and one day be key to disease control.

The Washington Wheat Commission applauds Pat Okubara, Tim Paulitz and Kurtis Schroeder for their groundbreaking research. No matter what type of farming practices growers use, be they conventional, reduced-tillage or direct-seed systems, root diseases affect growers' bottom lines. Developing ways to identify and measure soil pathogen populations are the first step towards improved disease control and breeding tolerant wheat varieties in the years to come.

Tim Paulitz, USDA-ARS researchplant pathologist