

GROWING KNOWLEDGE

Series content is coordinated by Dr. Lloyd Nackley, associate professor of nursery production and greenhouse management at Oregon State University in Corvallis, Oregon.



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Got plant problems?

Oregon State University Plant
Clinic is here to help diagnose



Entomologist and diagnostician, Bill Gerth, and clinic director, Dr. Mana Ohkura, evaluating a diagnostic sample. PHOTO BY CALEB FREEMAN

BY MANA OHKURA

Are you concerned your plants have a pest or pathogen problem because they have leaf spots, are chlorotic, or showing dieback? Are you noticing abnormal growth and you'd like to know what is causing it? The Oregon State University Plant Clinic is here to help diagnose your plant problems!

Understanding the cause of the problem is the first step in managing plant diseases and preventing them from spreading to healthy plants.

Plant problems largely fall into two categories: biotic problems, which are caused by living organisms such as insects,

fungi, viruses, or bacteria; and abiotic problems, which are caused by non-living factors, such as weather, chemicals, nutrient deficiencies, or mechanical injuries.

If the issue is biotic, identifying the pest or pathogen allows you to apply targeted control strategies, whether by applying an insecticide or fungicide, or by implementing new cultural practices. Some pathogens are vectored by arthropods or nematodes, so knowing the pathogen identifies which vector to control for.

If the issue is abiotic, identifying the causal factor allows you to modify cultural practices to prevent it from occurring again. The good news is you can avoid

unnecessary and costly spray applications if you were concerned a pest or pathogen was involved. This is also more environmentally sustainable; it reduces chemical inputs.

As many of you know, our former plant clinic director, Melodie Putnam, Ph.D., retired a couple of years ago, and I am fortunate to be inheriting such a successful clinic.

Putnam was at the forefront of adopting molecular diagnostic tools, such as polymerase chain reaction (PCR) and DNA sequencing, which greatly expanded testing capacity and the repertoire of pathogens the clinic can test for. I look forward to building on her work by



Growing Knowledge

offering new services that are useful to clients and by adopting new techniques for rapid and accurate testing.

To start, we have implemented several new PCR assays to expand our testing capability. For a list of testing services and information on how to submit a sample, please check out our website: bpp.OregonState.edu/Plant-Clinic.

Top 3 frequently asked questions

1. Does the clinic test soil for pathogens?

We offer soil testing for two major plant pathogens, *Verticillium dahliae* and *Phytophthora* species. *Verticillium dahliae* is the primary pathogen that causes *Verticillium* wilt on a wide range of hosts. *Phytophthora* species cause crown and root rot, stem cankers, and foliar blight on many plant species.

Please submit at least 2 cups of soil to test for either pathogen. A minimum of



Entomologist and diagnostician, Bill Gerth, examining a weevil larva under the microscope.
PHOTO BY CALEB FREEMAN

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five samples per acre is recommended for reasonable representation of a field.

For *V. dahliae* testing, please note that the assay takes approximately 4–5 weeks to complete. This is because the soil must be dried first, and then we culture for the pathogen. To reduce the turnaround time, we are actively working on optimizing a PCR-based assay, which we hope to implement next year.

For *Phytophthora* testing, we detect the pathogen by baiting with plant tissue, followed by culturing from the baits. Please note this method only detects *Phytophthora* species that produce swimming spores that are attracted to the baits under lab conditions. Typically, we identify the pathogen to genus level. To request species level identification, please contact us in advance to make special arrangements.

2. Does the clinic test irrigation water for pathogens?

We offer water testing for *Phytophthora* and *Pythium*, two common plant pathogens found in surface waters, such as rivers, streams, and lakes. These pathogens can spread through irrigation systems and often cause root diseases when soil moisture is high. Please submit 1–2 liters of water per sample. Testing is performed by using a filter to collect microbes in the water, and culturing them on selective media.

Typically, we identify *Phytophthora* and *Pythium* at the genus level. However, because these genera include many species that differ in host range and virulence, species level identification can be important when making management decisions. Therefore, in response to client interest, we are currently optimizing PCR-based assays to detect the following *Phytophthora* species in water samples: *P. cinnamomi*, *P. austrocedri*, and *P. ramorum*. If you are interested in testing for other specific species, please let us know and your input is welcome!

3. Does the clinic test for herbicide residue?

We do not test for herbicide residue. However, we do diagnose herbicide injury based on symptoms.



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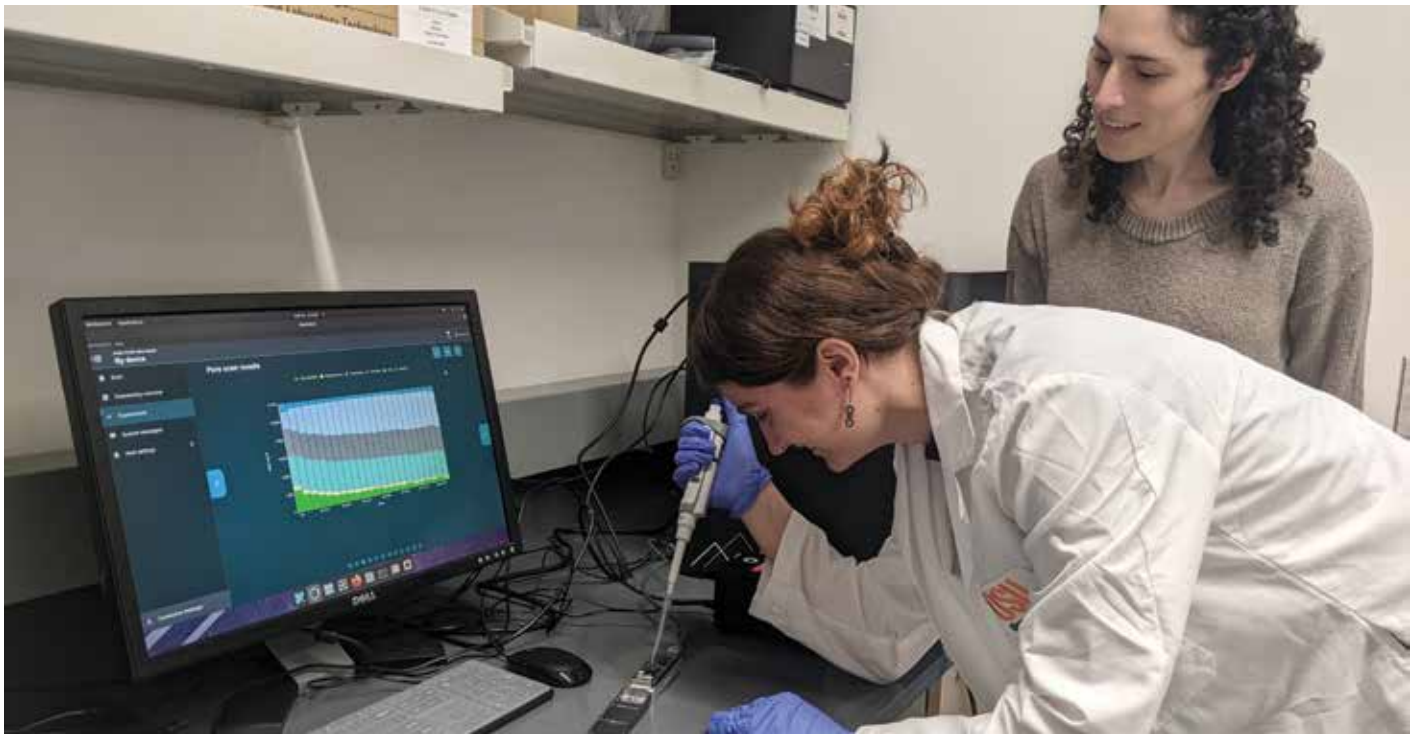
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Alexandra Weisberg, Ph.D. (right), and undergraduate student researcher Andrea Schiffer load metabarcoding samples onto a DNA sequencer. PHOTO COURTESY OF OSU PLANT CLINIC

New developments at the Plant Clinic

Supporting berry breeders with the Oregon Clean Plant Center: To support berry breeders in maintaining pathogen-free foundation stock, we are excited to announce our new partnership with the Oregon Clean Plant Center at the USDA-ARS Horticultural Crops Disease and Pest Management Research Unit (HCDPMRU; Corvallis, Oregon).

Through this collaboration, we now offer pathogen testing and foundation stock distributions for berry breeders as part of the National Clean Plant Network (NCPN). The effort is led by plant virologist, Dr. Cristian Olaya, and we are grateful for the support provided by USDA-ARS HCDPMRU and NCPN to launch this partnership.

Propagating from clean stock material is critical for economical and sustainable berry production, because many plant diseases cannot be cured. We test *Vaccinium*, *Rubus*, and *Fragaria* plants using qPCR, PCR, ELISA, and grafting techniques. Our testing panel includes viruses, phytoplasmas, and *Xylella fastidiosa*.

The chart on this page shows a list of commonly used services and associated fees. For more information on services offered through the Oregon Clean Plant Center, please contact us at TinyURL.com/PlantClinicCont.

DNA metabarcoding for faster test



Undergraduate student technician Kierstin Thompson prepares water samples for *Phytophthora* testing. PHOTO COURTESY OF OSU PLANT CLINIC

SERVICES	FEE
Set-up fee for PCR or ELISA testing	
A set-up fee is applied when fewer than 24 samples are submitted	\$275
End-point PCR testing	
PCR preparation fee per sample: Includes nucleic acid extraction, making cDNA (reverse transcription), and performing PCR with appropriate internal controls (Nad5).	\$88
PCR testing fee per virus/phytoplasma	\$33
ELISA testing	
ELISA fee per virus/phytoplasma	\$9
Graft indexing for <i>Rubus</i> and <i>Fragariae</i>	
Fee per bioindicator plant Plants will be grafted onto bioindicator plants and evaluated for symptoms 3 times	\$350

A list of commonly used testing services offered by the Oregon Clean Plant Center. These services are to test foundation stock through the National Clean Plant Network and are separate from the general diagnostic and testing services offered by the Plant Clinic..

results: Timely and accurate diagnosis is critical for controlling diseases before outbreaks occur and minimizing crop losses. At the Plant Clinic, we strive to maintain a consistent turnaround time, but we recognize that faster results are always beneficial.

To shorten our turnaround time, we are exploring the use of a DNA sequencing method known as DNA metabarcoding, in collaboration with Alexandra Weisberg, Ph.D., and Nik Grünwald, Ph.D. (USDA-ARS), in the Botany and Plant Pathology Department. With support from the Agricultural Research Foundation, we are conducting preliminary research to evaluate the accuracy and sensitivity of this technique.

DNA metabarcoding begins with extracting total DNA from a sample, which includes both plant DNA and DNA of the abundant microbes within the plant. Then, a specific region of microbial DNA — also known as a barcode — is amplified and sequenced. The resulting DNA sequences from the microbes are then analyzed computationally to identify the microbial species present in the sample.

A major advantage of DNA metabarcoding is its ability to detect many microbial species in a single assay. This makes it possible to process multiple diagnostic samples simultaneously, even if they are infected with different pathogens.

In contrast, most PCR assays are designed to test a single pathogen at a time, which limits throughput and efficiency. If proved to be practical, DNA metabarcoding could significantly increase testing throughput. Ultimately, this would lead to faster test results and enable growers to take management actions earlier. ©

Mana Ohkura is Oregon State University Plant Clinic Director and assistant professor of practice. Reach her at OhkuraM@OregonState.edu.



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