Series content is coordinated by Dr. Jay Pscheidt, professor of botany and plant pathology at Oregon State University in Corvallis, Oregon.

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Heroes and villains

Research identifies harmful and beneficial microbes in nursery soil

BY JERRY WEILAND, CAROLYN SCAGEL, NIK GRUNWALD, ZACHARY FOSTER, VAL FIELAND, AND LUISA SANTAMARIA

FOUR YEARS AGO, we received funding from the Floriculture and Nursery Research Initiative to study the interaction of soil-borne pathogens and environment on nursery plant health and disease control (*Digger*, July 2015).

Since then, we have made significant progress in identifying the beneficial and pathogenic microbes (microscopic fungi and oomycetes like *Phytophthora* and *Pythium*) found in rhododendron roots and in assessing how pathogen diversity affects disease development and control.

Our initial research focus has been on rhododendron because it is a common ornamental crop here in the Pacific Northwest nursery industry and because many of the soil-borne pathogens that affect rhododendron also affect other plant species.

What we learn about these pathogens on rhododendron will help us solve root diseases on other horticultural crops affected by the same pathogens.

Our research team consists of experts in nursery production, plant pathology, mycorrhizae, plant physiology, and extension services from the Horticultural Crops Research Laboratory (USDA-ARS), Oregon State University, and Oklahoma State University. Together, we hope to provide a comprehensive approach to identifying the key beneficial and pathogenic microbes that affect nursery plant production and to evaluate how that microbial diversity impacts disease control.

Our goal is to improve disease management and soil health for nursery production in the Pacific Northwest. Here, we will summarize what we have discovered so far about microbial diversity and how soil-borne pathogen diversity might be affecting disease control.

Rhododendrons with root rot in a low-lying area of the field. PHOTO BY JERRY WEILAND

Microbial diversity research

We are using a technique called metabarcoding to study the diversity of microscopic fungi and oomycetes (the microbiome) that occur in the roots of healthy rhododendrons from different nurseries. Some of these microbes are beneficial (e.g., mycorrhizae), some are neutral decomposers of organic matter, and some are plant pathogens (e.g., *Pythium* and *Phytophthora* species).

By comparing the rhododendron microbiome at several different nurseries from both field-grown and potted plants, we expect to identify organisms that are consistently associated with plants and might be candidates for biological control or provide plant growth promoting effects. We also expect to identify common root pathogens, as well as previously unrecognized pathogens, that are important players in reducing root health.

Our preliminary results suggest

Heroes and villains

that each nursery has a distinct community of fungi and oomycetes, although a few specific organisms are common among all nurseries, including fungi typically thought to be mycorrhizal, the fungus *Trichoderma* (sometimes used as a biocontrol agent), and several saprophytic species that decompose organic matter.

Pathogens were detected, but none were consistently found across all nurseries. Although we found that field-grown plants had different microbial communities than potted plants, those communities were not necessarily more diverse.

We also looked to see whether there were any differences in community diversity between three rhododendron cultivars, but did not find any. This is surprising because similar studies on other crops have revealed differences in the root microbiome between closely related cultivars.

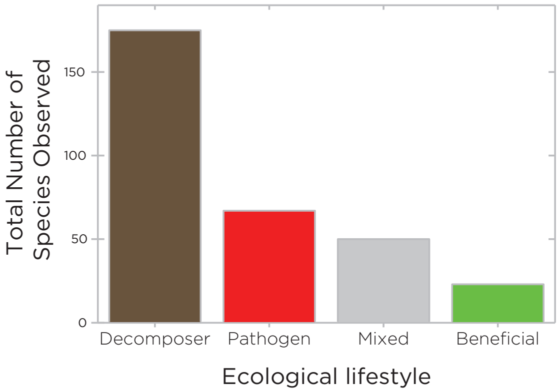
Finally, we tried to assign the organisms we found to different ecological lifestyles and estimated that most were decomposers, some were pathogens or had multiple possible lifestyles (mixed), and relatively fewer were beneficial. However, the beneficial organisms, while not as diverse, were some of the most commonly found organisms in our study.

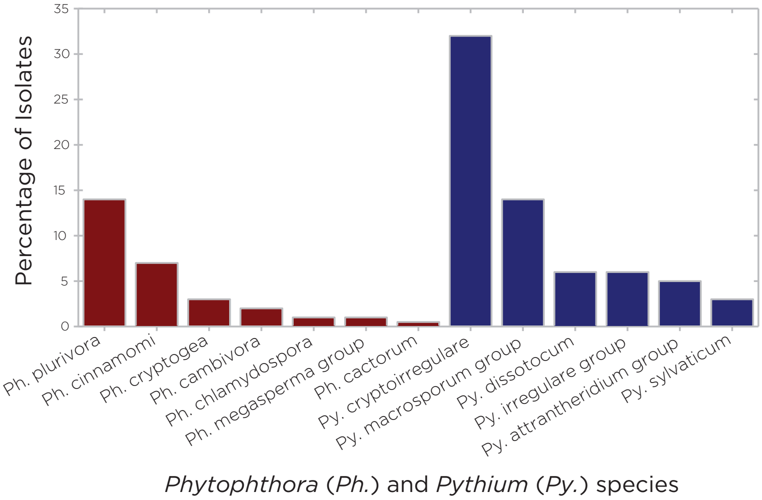
Soil-borne pathogen diversity

In addition to our microbiome work on healthy plants, we are surveying diseased rhododendrons to find out which soil-borne pathogens are the most common and cause the most damage.

From 2013 to 2017, we sampled rhododendrons with symptoms of root rot from seven cooperating nurseries that grow rhododendron. So far, *Phytophthora cinnamomi*, *Phytophthora plurivora*, and *Pythium cryptoirregulare* are the most common pathogens isolated. The only pathogen we found at all seven nurseries was *Pythium cryptoirregulare*, so it was important that we assess the ability of this pathogen to cause disease.

The good news is that, although *Pythium cryptoirregulare* is common, it does not appear to be very aggressive





Left: Inferred lifestyles of fungi and oomycetes found in roots of healthy looking rhododendron nursery plants. Right: Common soil-borne pathogens associated with root rot in rhododendron nursery plants.

on rhododendrons with established root systems. We tested the ability of *Pythium cryptoirregulare* to cause root rot on 6- to 8-month-old rooted rhododendron cuttings and found that, after three months, it was only occasionally able to cause enough damage to reduce plant size.

P. cinnamomi and *P. plurivora*, on the other hand, were also common, but were only found at six of the seven nurseries. In contrast to *Pythium cryptoirregulare*, both of these *Phytophthora* species caused extensive root rot in the same amount of time, especially when there were high amounts of inoculum in the soil. However, even at low levels, *P. cinnamomi* was able to reduce growth in inoculated rhododendron plants.

To better understand the variability within these three pathogens, we are using a technique called genotyping-by-sequencing to provide us with a genetic fingerprint for each individual. Current results suggest that although *P. plurivora* populations are moderately diverse, they tend to be very similar across different nurseries. This indicates that *P. plurivora* is common in the nursery industry and likely moves readily among nurseries on infected, but asymptomatic nursery stock.

We are also studying the genetic diversity in *P. cinnamomi* and *Pythium cryptoirregulare*. This work will form »

Roots often fail to grow out of the original root ball with *Phytophthora* root rot.
PHOTO BY JERRY WEILAND

the basis for developing detection assays to help diagnostic laboratories identify these pathogens. Results will also reveal whether the pathogens reproduce clonally or sexually, which can help us understand how likely the pathogens will be able to overcome fungicide or cultivar resistance, and how pathogens move among nurseries.

Disease control

We have started to evaluate chemical options for managing root rot, including fungicides and gypsum. For the fungicide work, we are investigating how pathogen diversity affects disease control efficacy.

We compared the ability of mefenoxam and phosphorous acid to control root rot caused by either *P. cinnamomi* or *P. plurivora* on *R. catawbiense* cultivars. We applied the fungicides to either the leaves as a foliar spray or to the roots as a soil drench.

For *P. cinnamomi*, only the fungicide soil drenches were effective at reducing root rot. Foliar applications of either product were ineffective. This makes sense because the soil is where these root pathogens are located and causing damage. However, for *P. plurivora*, neither fungicide was effective in controlling root rot, regardless of how they were applied.

These results must be viewed with caution. We may have, by chance, inadvertently selected a fungicide-resistant isolate of *P. plurivora*. While the isolate of *P. plurivora* used in our research came from a diseased nursery plant, and our diversity studies suggest *P. plurivora* has low population diversity in Oregon nurseries, other *P. plurivora* isolates may be more sensitive to these fungicides. Therefore, we are evaluating a larger number of *P. cinnamomi* and *P. plurivora* isolates to gain more confidence in our findings.

With gypsum, we evaluated whether application (0, 1, 5 or 10 percent) to soil was able to control root rot in young *Rhododendron* 'Looking Glass' H-2 plants inoculated with *P. plurivora* or *P. cinnamomi*. In this preliminary trial, no significant visible symptoms of root rot were present on plants, possibly because the cultivar may be resistant to the pathogen isolates we used. Gypsum application at 5 and 10 percent did, however, improve plant growth compared to plants grown in substrate with 1 percent or no gypsum.

Future research

The next stage of our research is to continue to evaluate the effect of environment on the development of root rot. We have ongoing studies to evaluate the effect of irrigation and plant nutrition on root rot severity. In addition, using the tools we have developed to assess microbial diversity, we will be evaluating how cultural practices and disease control measures alter the soil microbial community in nursery systems.

We are currently conducting an experiment to test the effect of common root rot pathogens on the composition of fungal communities in the roots of rhododendron. Is the non-pathogenic community involved in pathogen establishment or is disease purely an interaction between the host plant and the pathogen? Do different pathogens interact with the non-pathogenic community differently?

We are also considering an experiment testing the effect of common fungicides on the non-pathogenic community. It has been demonstrated in many studies that members of the non-pathogenic community can provide nutrients (e.g., mycorrhizae) or protection from disease (e.g. *Trichoderma*). Do common fungicides kill these beneficial organisms as well as the pathogens? Could protecting plants with fungicides that also kill symbionts make them more susceptible to pathogens in the future and therefore disease control would become more reliant on continued fungicide application?

Discovering interactions between beneficial/neutral organisms and pathogens could inform future disease control strategies.

The results from our studies will be used to establish criteria for implementing disease control strategies in nursery production systems. One continuing challenge for nursery growers in effectively managing disease is proper pathogen identification. We are hoping to address this challenge in future research by developing simple diagnostic tools which can be used by growers and plant disease diagnosticians to better target disease control measures.

Dr. Jerry Weiland (Plant Pathology), Dr. Nik Grunwald (Plant Pathology), and Dr. Carolyn Scagel (Plant Physiology) are research scientists with the Horticultural Crops Research Laboratory, USDA Agricultural Research Service in Corvallis, OR and are also affiliated with the Departments of Botany and Plant Pathology or Horticulture at Oregon State University. They can be reached at Jerry.Weiland@ars.usda.gov, Nik.Grunwald@ars.usda.gov, *and* Carolyn.Scagel@ars.usda.gov). *Zachary Foster is a Ph.D. student and Val Fieland is a Biological Sciences Technician in Nik Grunwald's laboratory. Dr. Luisa Santamaria (Botany and Plant Pathology) is an associate professor at Oregon State University and the North Willamette Research and Extension Center. She can be reached at* luisa.santamaria@oregonstate.edu.