Pathology Smorgasbord: Biocontrol, Pathogen Movement, and Recent Fumigation Results

Jerry E. Weiland

Research Plant Pathologist, U.S. Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Laboratory, Corvallis, OR

Abstract

This article summarizes recent research on soilborne pathogens, disease control, and new forest diseases including reduced-rate soil fumigation, *Pythium* diversity and biocontrol, pathogen movement among nurseries, and a new incense-cedar disease. Results from the fumigation study indicate that reduced-rate soil fumigation is effective for soilborne disease and weed control. Results from the biocontrol study provide a partial explanation for why biocontrol may sometimes fail in forest nurseries, and results from the population genetics study show that *Pythium* species are being moved in the forest nursery industry. Our latest forest tree research found a new incense-cedar canker disease in Oregon. This paper was presented at the joint annual meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers' Association (Troutdale, OR, September 14–15, 2016).

Figure 1. Damping-off of a young Douglas-fir seedling caused by *Pythium* species. (Photo by Jerry Weiland, 2010)

Figure 2. Root rot of 1-year-old Douglas-fir seedlings in nursery beds where water collects at the end of a row. (Photo by Jerry Weiland, 2010)

Introduction

Soilborne pathogens and weeds are important production constraints for the forest nursery industry. Some of the most common soilborne pathogens are species of *Pythium*, *Fusarium*, and *Cylindrocarpon*. Together, these pathogens cause damping-off (figure 1) and root rot (figure 2) of seedlings, resulting in chlorosis, stunting, and seedling death. The variety of soilborne pathogens affecting forest nurseries makes it difficult to achieve adequate disease control in the absence of soil fumigation. For example, the fungicides used to control *Pythium* are mostly ineffective against *Fusarium* and *Trichoderma*. Species diversity within each of the three pathogen genera may also affect disease control. Nineteen *Pythium* species have been found in the forest nurseries of Washington and Oregon. Not only do these species differ in their ability to cause disease, but also in how they respond to fungicides and biocontrol agents. New evidence shows that *Pythium* species and fungicide-resistant *Pythium* isolates have been moved among nurseries, which further complicates disease control decisions. This highlights the risk for accidentally introducing new pathogens and diseases into locations where they previously did not occur.

Figure 3. Soil fumigation at a forest nursery. The fumigant is injected into the soil and then covered with totally impermeable film (TIF) to keep the fumigant in place. (Photo by Jerry Weiland, 2010)

Traditionally, control of soilborne pathogens and weeds is achieved in forest nurseries through soil fumigation with methyl bromide and chloropicrin (figure 3). The benefit of soil fumigation is that fumigants have broad-spectrum activity against most pathogen species compared with the more targeted effects of fungicides and biocontrol agents. Methyl bromide is being phased out through the Montreal Protocol, however, and it is uncertain how much longer the industry will have access to this fumigant. In addition, current Environmental Protection Agency (EPA) regulations require a buffer zone (nonfumigated area between fumigated fields and neighboring properties) that can be reduced based on certain conditions, including tarping and the amount of fumigant applied. In general, the more impermeable the tarp (e.g., totally impermeable film, or TIF), and the less fumigant that is applied, the smaller the buffer zone. It is unknown, however, whether reduced-rate fumigant applications (fumigants applied below the label rate) will be effective for disease and weed control. Therefore, forest nursery managers are interested in fumigant alternatives to methyl bromide and in the efficacy of reduced-rate fumigants against soilborne pathogens and weeds.

Reduced-Rate Soil Fumigation

In 2010 to 2012, a reduced-rate soil fumigation study (Weiland et al. 2016a) was established to evaluate the effects of three fumigant treatments: (1) MBC, 50/50 methyl bromide/chloropicrin at 250 lb/ac (280 kg/ha); (2) MSC, metam sodium plus chloropicrin at 27 gal/ac (253 l/ha) plus 150 lb/ac (168 kg/ha); and (3) DPC, 40/60 1,3-dichloropropene/chloropicrin at 285 lb/ac (319 kg/ha), as well as a nonfumigated (NF) control. The three fumigant treatments were applied in August 2010 at a rate requiring a 25-ft (7.6-m) buffer zone, according to 2010 EPA guidelines. Each treatment was replicated four times, and the experiment was repeated at two nurseries. One-year-old, bareroot 1 + 0 Douglas-fir seedlings were transplanted into each nursery, in May of the following year. Approximately 10 months after fumigation, four biocontrol treatments were applied to seedlings within the MSC, DPC, and NF treatments to see if disease control could be improved after fumigation. The four treatments were: (1) *Streptomyces lydicus* (6 oz/100 gal [47 ml/100 L]) plus *Bacillus subtilis* (64 oz/100 gal [500 ml/100 L]); (2) *Trichoderma harzianum* (5 oz/100 gal [39 ml/100 L]) plus *Gliocladium virens* (2 lb/100 gal [2400 g/100 L]); (3) All four biocontrol agents applied in combination; and (4) no biocontrol treatment (water only). The biocontrol treatments were applied three times during the growing season in June, July, and October 2011.

Results showed that all three fumigant treatments were effective in reducing soilborne pathogen populations (*Pythium* and *Fusarium*) in the soil (data not shown) and on seedling roots (figure 4 for *Pythium*), in comparison to the NF plots. None of the biocontrol treatments were effective, however (data not shown). Weeds were also controlled by all three fumigant treatments (figure 5) relative to the NF plots. Seedlings were largest and healthiest from the MBC and MSC treatments, and were the smallest and least healthy in the NF plots (data not shown). Seedlings in NF plots were on average 3 to 5 in (7 to 13 cm) shorter than those in fumigated plots. Results from this study show that reduced

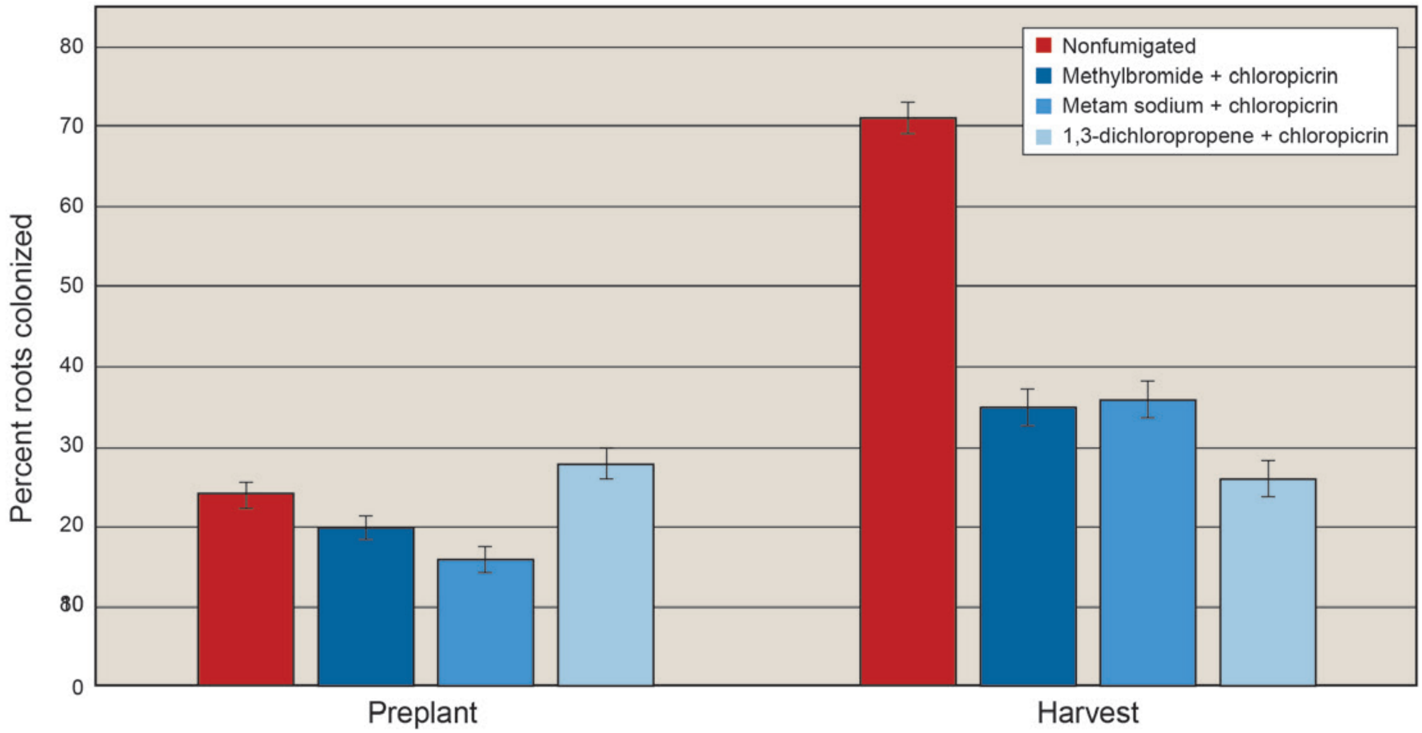

Figure 4. Average percent root colonization by *Pythium* species of 1-0 Douglas-fir seedlings before planting into treatment plots in mid-May 2011 (preplant) and at harvest in December 2011 (harvest) across two nurseries. Results were similar for *Fusarium* species (not shown). Error bars = standard error.

rates of these fumigants help produce healthy seedlings and are effective for managing soilborne diseases and weeds in forest nurseries.

Pythium Species Diversity and Biocontrol

In 2008, a study was conducted to describe the diversity of *Pythium* species in forest nurseries of Oregon and Washington (Weiland 2011). Soil at three forest nurseries (two in Oregon and one in Washington) was surveyed, and 300 individuals of *Pythium* were identified from each nursery. The results showed that each nursery had a different set of *Pythium* species. For example, the most commonly identified species at each nursery was different (table 1): *P. irregulare* was the most common at nursery A, whereas *P. 'vipa'* and *P. dissotocum* were more common at nursery B and C, respectively. Additionally, although *P. irregulare* occurred at all three nurseries, it made up a different percentage of the population at each nursery (65 percent at nursery A, 10 percent at nursery B, and 6 percent at nursery C). Finally, some species were only present at a single nursery. For example, *P. 'vipa'* was only found at nursery B.

This *Pythium* species diversity makes a difference in terms of disease (Weiland et al., 2013). In a greenhouse pathogenicity study with Douglas-fir seedlings (figure 6), eight *Pythium* species were found to be weak pathogens causing root lesions and less than 25

Table 1. Percentage occurrence of the five most common *Pythium* species at three forest nurseries.

Nursery A (WA)		Nursery B (OR)		Nursery C (OR)	
%	**Species**	**%**	**Species**	**%**	**Species**
65	*P. irregulare*	53	*P. 'vipa'*	47	*P. dissotocum*
10	*P. torulosum*	15	*P. aff. macrosporum*	14	*P. ultimum*
6	*P. aff. macrosporum*	10	*P. irregulare*	11	*P. aff. spiculum*
6	*P. irregulare* type III	9	*P. sylvaticum*	8	*P. sylvaticum*
5	*P. aff. spiculum*	8	*P. ultimum*	6	*P. irregulare*
92% of total population		**95% of total population**		**86% of total population**	

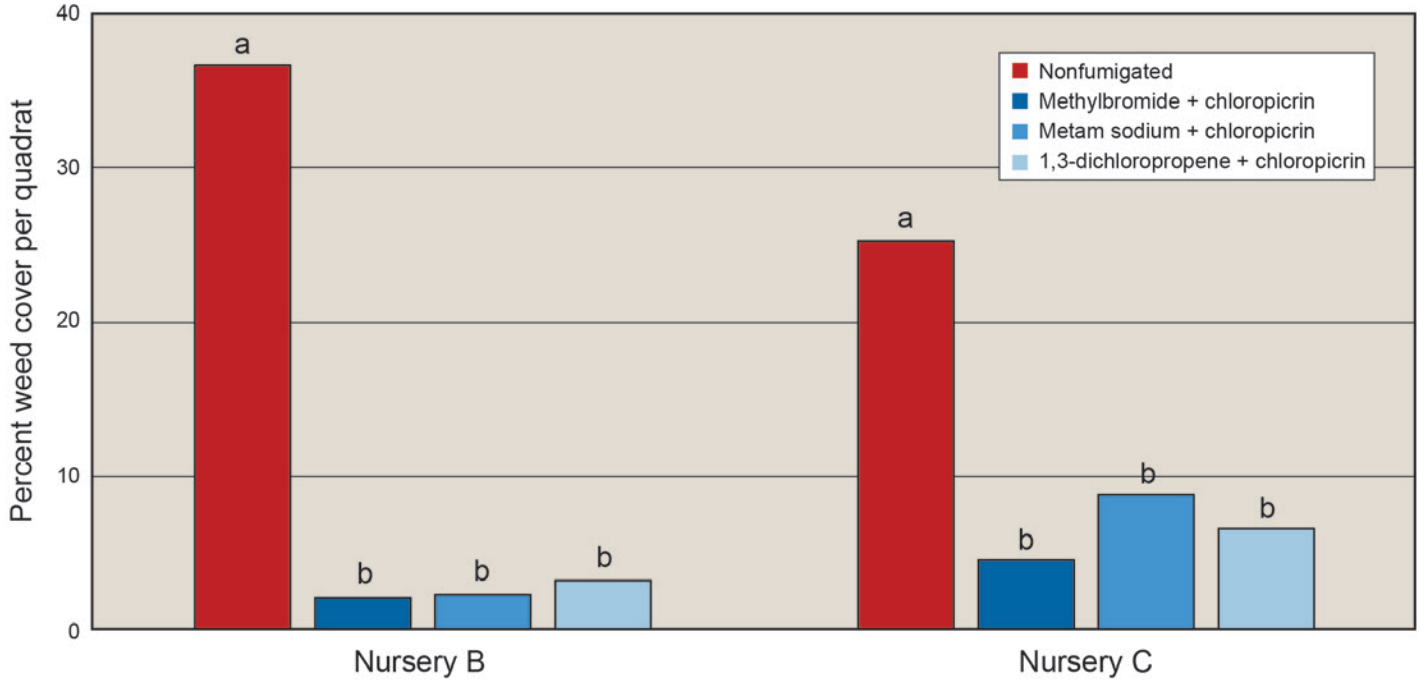

Figure 5. Effect of treatment on average weed cover (percent) at both nurseries in January 2012.

percent seedling mortality, compared to noninoculated control plants (figure 7). Eight other *Pythium* species were considered aggressive pathogens, however, and caused greater than 25 percent seedling mortality (figure 8). Revisiting the *Pythium* species diversity research from Weiland 2011, the percentage of aggressively pathogenic *Pythium* species can now be identified at each of the three forest nurseries (table 2). These results show that 76 percent of the *Pythium* population at nursery A and 85 percent of the population at nursery C are aggressive pathogens compared to only 43 percent at nursery B. Although direct evidence is not yet available, this might be an indication that the disease pressure due to *Pythium* may be almost one-half at nursery B, compared with the other two nurseries. Given the diversity of *Pythium* species in the soil at each nursery, is it reasonable to expect that a single biocontrol agent will work equally well against all *Pythium* species? To test this, we evaluated 16 *Pythium* species against a commercial Streptomyces lydicus strain in a Petri plate assay (figure 8, Weiland 2014).

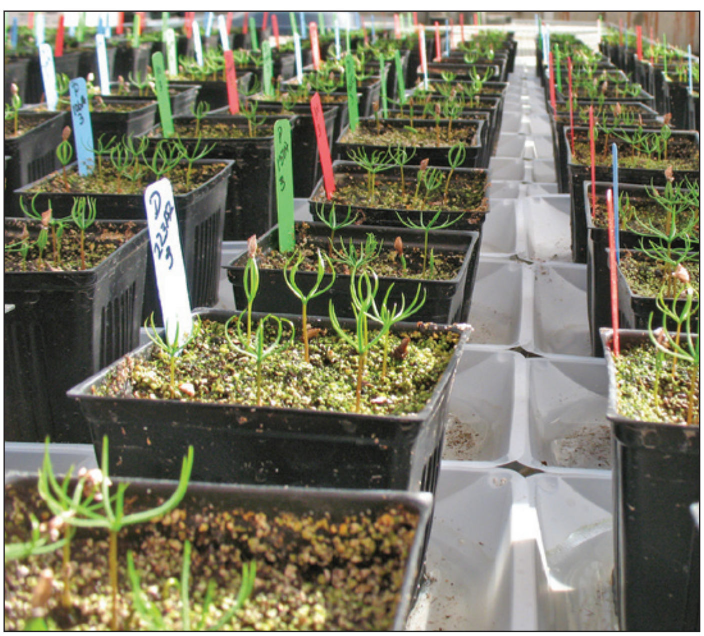

Figure 6. Douglas-fir seedlings inoculated with 16 different *Pythium* species in a greenhouse pathogenicity assay. (Photo by Jerry Weiland, 2011)

Table 2. Percentage occurrence of eight aggressively pathogenic Pythium species at three forest nurseries.

Pythium species	Nursery A (%)	Nursery B (%)	Nursery C (%)
P. dissotocum	2	0	47
P. irregulare	65	10	6
P. aff. macrosporum	6	15	7
P. mamillatum	1	0	2
P. aff. oopapillum	0	0	1
P. rostratifingens	1	1	0
P. sylvaticum	0	9	8
P. ultimum	1	8	14
Total	76	43	85

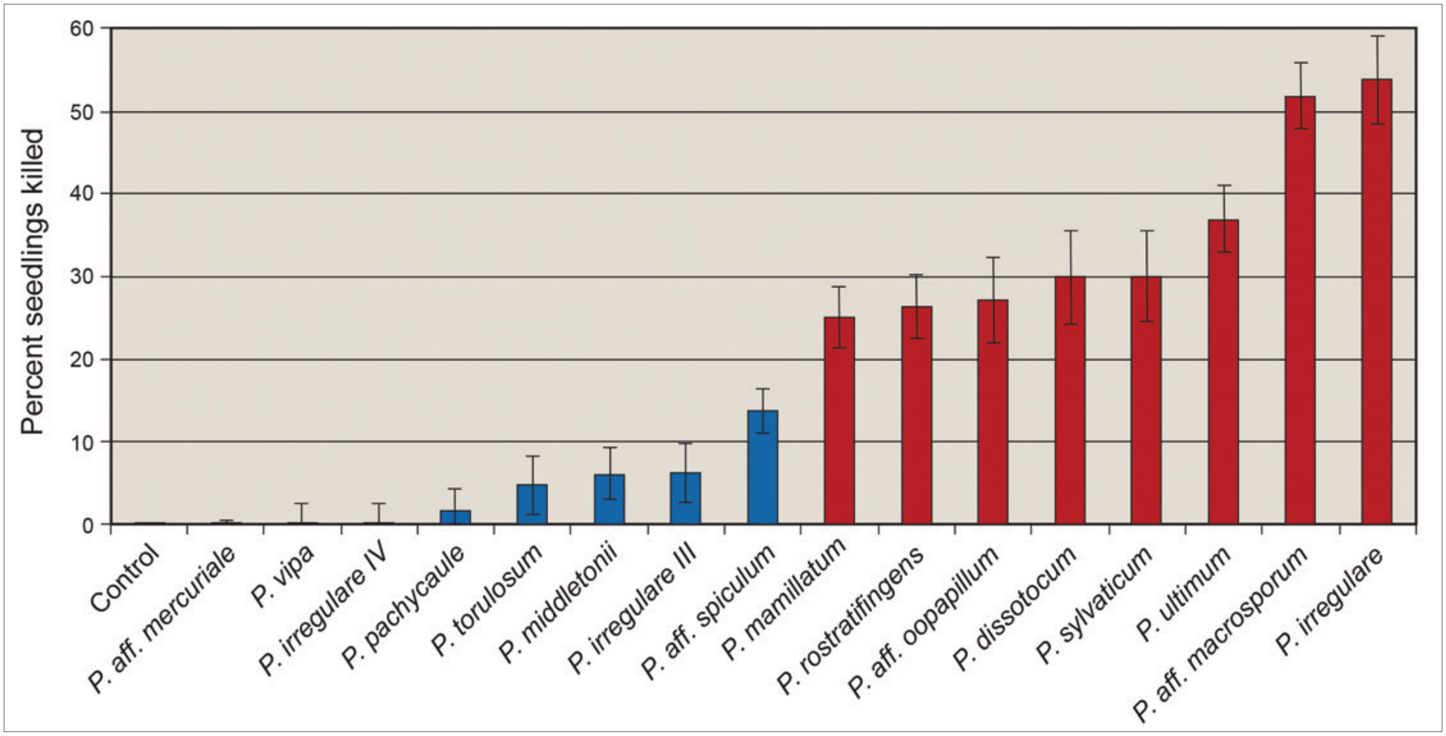

Figure 7. Percent Douglas-fir seedlings killed by eight weakly pathogenic *Pythium* species (less than 25 percent mortality, on left in blue) and eight aggressively pathogenic *Pythium* species (more than 25 percent mortality, on right in red) compared to noninoculated seedlings (control, far left).

Results indicated that the biocontrol agent inhibits each *Pythium* species to a different degree; some species, like *P. torulosum*, were inhibited more by *S. lydicus* than other species, like *P. ultimum* (figure 9). In addition, we found that the nursery from which the *Pythium* species were isolated affected the results. For example, individuals of *P. irregulare* from nursery A were inhibited more than individuals of the same species from nursery B (data not shown). These results show that *S. lydicus* does not work equally against all *Pythium* species and may partially explain why applications of biocontrol in forest nurseries may sometimes fail against soilborne pathogens. Poor biocontrol establishment, poor environmental conditions, and improper application, however, are among the explanations for why a biocontrol can fail. Nevertheless, these results suggest it may be too much to expect that a single biocontrol agent will be effective against the diversity of *Pythium* species in the soil, not to mention the diversity of soilborne *Fusarium* and *Cylindrocarpon* species that were not even tested in our study.

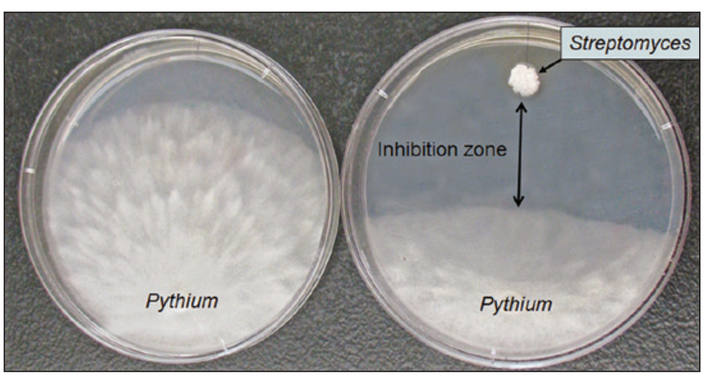

Figure 8. Petri plate assay to evaluate *Pythium* species inhibition by the biocontrol agent *Streptomyces lydicus*. No inhibition was observed in plates only containing a *Pythium* isolate (left). Plates containing *S. lydicus* inhibited the growth of *Pythium* toward the other side of the plate, creating an inhibition zone (right). (Photo by Jerry Weiland, 2013)

Pathogen Movement Among Nurseries

In 2015, a study evaluating the genetic relatedness of *Pythium* species (*P. irregulare*, *P. ultimum*, and *P. sylvaticum*) from three forest nurseries was conducted to find out if these pathogens are being moved among nurseries (Weiland et al. 2015). For *P. irregulare*, individuals at nursery A were genetically identical or very similar to those from nursery B and C, indicating that this species had been moved among the three nurseries. Similar results were found with *P. ultimum* and *P. sylvaticum* from nurseries B and C. In addition, two fungicide-resistant (mefenoxam) individuals of *P. ultimum* were found, one each at nursery B and C. These two resistant individuals were genetically related, which provided evidence that fungicide-resistant species are also being moved among nurseries. It

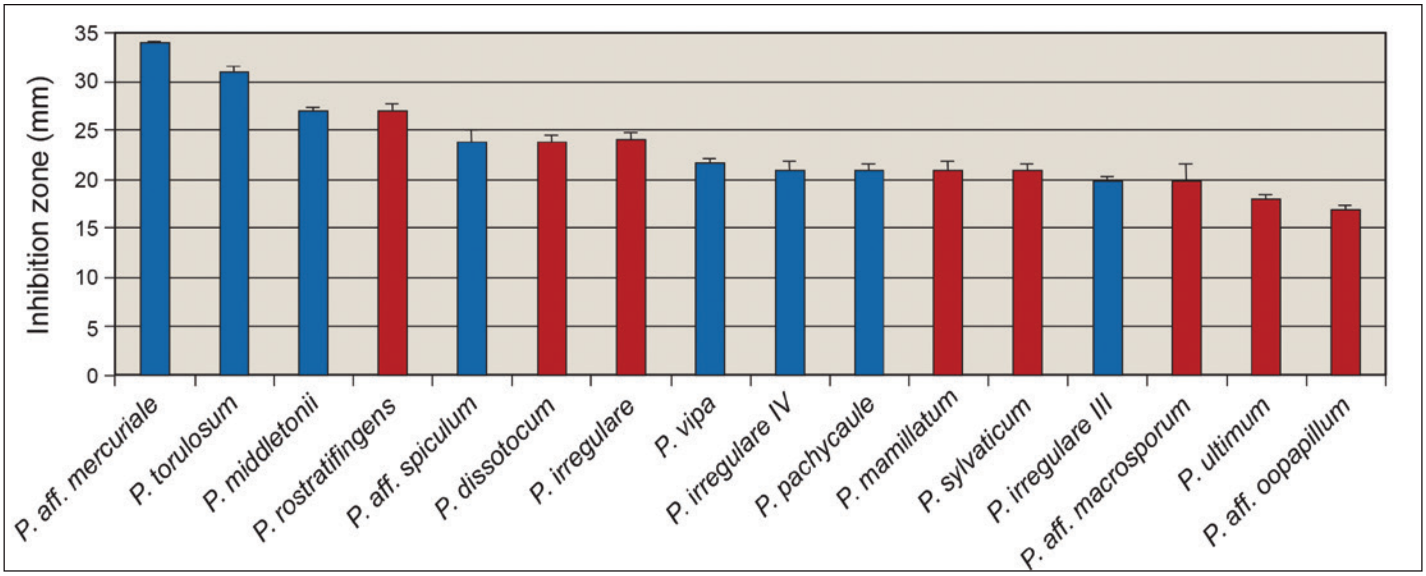

Figure 9. Inhibition of 16 *Pythium* species by Streptomyces lydicus strain WYEC108, a commercial biocontrol agent. Species on the left are inhibited more by the *S. lydicus* than those on the right. Species in blue are considered weakly pathogenic (less than 25 percent mortality) and those in red are considered aggressive pathogens (more than 25 percent mortality).

is unknown whether *Pythium* is being moved from nursery to nursery on nursery stock or on equipment, but moving new species or fungicide-resistant individuals to nurseries where they did not occur previously is risky. For example, *P. dissotocum* was not found at nursery B (table 2), therefore, this nursery would be at risk of importing this pathogen if it received nursery stock or shared equipment from nurseries A or C. Likewise, nursery A would be at risk of accidentally importing fungicide-resistant isolates of *P. ultimum* from nurseries B and C. Nursery managers could help reduce this risk by designating specific fields for seedlings imported from other nurseries and then reserving the remaining field space for inhouse seedling production. Although difficult, shared equipment should also be cleaned whenever possible. Future research directions might focus on whether fungicides or fumigation can be used to limit the spread and establishment of soilborne pathogens in the industry.

New Incense-Cedar Disease

A new incense-cedar (*Calocedrus decurrens* [Torr.] Florin) disease was recently discovered in the Willamette Valley of Oregon (Weiland et al. 2016b). The pathogen, *Phaeobotryon cupressi*, causes branch cankers that are scattered throughout the crown of the tree. Infected branches die, thereby destroying the tree's ornamental value (figure 10). Interestingly, the pathogen was first discovered causing branch cankers of Italian cypress in Iran in 2009. Prior to its discovery in Oregon in 2015, it was described only once from a healthy juniper in Kansas. Similar symptoms have been reported on native populations of incense-cedar in the Cascade Mountains, but the pathogen has not been confirmed as causing disease at those locations. We have not heard of similar symptoms occurring in forest nurseries, but this disease may be something to keep in mind for anyone producing this native tree species.

Figure 10. Incense-cedar with branch cankers caused by *Phaeobotryon cupressi*. (Photo by Jerry Weiland, 2014)

Conclusions

In the short term, soil fumigation with methyl bromide will continue to play a large role in producing healthy forest seedlings. As methyl bromide stocks are depleted, however, the industry will need to switch to other fumigant chemistries. Current studies show that reduced-rate fumigant treatments can be effective for controlling soilborne pathogens and weeds. What remains unknown is how long control will last after application, in comparison to the standard application of methyl bromide:chloropicrin (67:33) at 350 lb/ac (392 kg/ha). Studies are needed to see how many crop cycles can profitably be produced, following newer alternative soil fumigation treatments, without compromising seedling quality. Fungicides will continue to play an important role in providing supplemental disease control. The development of fungicide resistance is a concern, however, and growers must alternate fungicide chemistries to prevent the further spread of resistance in the industry. Biocontrol options for soilborne pathogens remain limited and inconsistent. It is unlikely that a single biocontrol agent will provide adequate disease control, given the diversity of soilborne pathogens that are present in forest nurseries. Further research on combining biocontrol agents may be useful, but it will probably take many years before effective formulations become available. Finally, growers must be wary of accidental pathogen introductions. The awareness of pathogen movement is growing in all nursery industries, particularly when new diseases and insects are introduced that devastate our native tree species.

Address correspondence to—

Jerry E. Weiland, USDA-ARS, Horticultural Crops Research Laboratory, 3420 NW Orchard Ave, Corvallis, OR, 97330; email: jerry.weiland@ars.usda.gov; phone: 541–738–4062.

Acknowledgments

The author thanks forest nursery research collaborators Bryan Beck, Anne Davis, and Nik Grunwald (USDA-ARS); John Browning, Glenn Cattnach, Carol Larson, and Will Littke (Weyerhaeuser Company); Mike Conway and Jeff Fowler (Trident Ag Products); Robert Edmonds (University of Washington); Tim Miller (Washington State University); Patricia Garrido and Carla Garzon (Oklahoma State University); Zhian Kamvar, Melodie Putnam, Maryna Serdani, and Michelle Wiseman (Oregon State University), and Richard Sniezko (USDA Forest Service).

REFERENCES

Weiland, J.E. 2011. Influence of isolation method on recovery of *Pythium* species from forest nursery soils in Oregon and Washington. Plant Disease. 95: 547–553.

Weiland, J.E. 2014. *Pythium* species and isolate diversity influence inhibition by the biological control agent Streptomyces lydicus. Plant Disease. 98: 653–659.

Weiland, J.E.; Beck, B.R.; Davis, A. 2013. Pathogenicity and virulence of *Pythium* species obtained from forest nursery soils on Douglas-fir seedlings. Plant Disease. 97: 744–748.

Weiland, J.E.; Garrido, P.; Kamvar, Z.N.; Espindola, A.E.; Marek, S.M.; Grunwald, N.J.; Garzon, C.G. 2015. Population structure of *Pythium* irregulare, P. ultimum, and P. sylvaticum in forest nursery soils of Oregon and Washington. Phytopathology 105: 684–694.

Weiland, J.E.; Littke, W.R.; Browning, J.E.; Edmonds, R.L.; Davis, A.; Beck, B.R.; Miller, T.W. 2016a. Efficacy of reduced rate fumigant alternatives and methyl bromide against soilborne pathogens and weeds in western forest nurseries. Crop Protection 85: 57–64.

Weiland, J.E.; Sniezko, R.A.; Wiseman, M.S.; Serdani, M.; Putnam, M.L. 2016b. First report of *Phaeobotryon cupressi* causing canker of *Calocedrus decurrens* (Incense-cedar) in Oregon. Plant Disease. 100: 1793.