Center for Advanced Forestry Systems
2020 Annual Meeting Project Progress Report

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YEAR: 3 of 5
PROJECT TITLE: Improving White Pine Seedling Survival by Combining Blister Rust Resistance with Defense-enhancing Endophytes

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PROJECT DESCRIPTION: Western white pine (WWP) is among the most productive and valuable species on moist sites in the inland Northwest. However, an exotic fungal disease, white pine blister rust (WPBR), has devastated natural populations of WWP since its introduction in the early 1920s (Fins et al, 2001). In the 1950s, the USDA Forest Service launched a WPBR breeding program designed to capture the genetic resistance found in some white pine trees in heavily infected stands (Bingham, 1983). As a result, resistant seed sources were developed and widely planted in the Inland Northwest (Mahalovich 2010). Whereas those seed sources are more resistant than unimproved seed sources, significant levels of WPBR infection and mortality still occur on some sites.

In the present study, we were investigating if endophytes with antagonistic properties to Western white pine seeds and seedlings could improve genetic resistance. Two different mechanisms of genetic resistance to the blister rust were utilized. The first mechanism is known as the bark reaction. As the blister rust travels down the plant from the point of infection (needles), cankers form on the bark. The bark reaction resistance mechanism involves the formation of wound-periderm around cankers in the bark. By forming this tissue, the tree is compartmentalizing the fungus and preventing its spread (Hoff, 1986). The second mechanism is referred to as short shoot resistance. Rust spores enter the needle through the stomata and continue growth down the needle towards the short shoot. Trees possessing short shoot resistance are able to halt the growth of the fungus before or very shortly after it reaches the trunk. In other words, once the fungus reaches the short shoot, the resistance mechanism prevents further pathogen growth. Additionally, the mycelium already present in the needle will begin to die, and this necrotic reaction will radiate outwards towards the tip of the needle (Hoff & McDonald, 1971). After treatment with endophytes, the trees were inoculated with *Cronartium ribicola* in Sept. 2018. **Endophytes.** Six endophytes were selected for this study. Four of these endophytes were isolated from Western white pine seed and demonstrated promising antagonistic behavior *in agaro.* Two of these seed endophytes are fungi; one is in the *Penicillium yarmokense-arizonense* species complex (CBS #144778) and the other is a new *Penicillium* species (CBS #144780) related to *Penicillium raistrickii* and *Penicillium sajarovii.* The remaining two seed endophytes are bacterial isolates. One bacterium was identified as *Bacillus velezensis*. Bacillus is a genus of bacteria that contains members known to promote plant growth. The final endophyte is a *Streptomyces* species. *Streptomyces* members produce numerous antibiotics; the number of antimicrobial compounds this genus could potentially produce may be as high as 150,000 (Watve et al., 2001)!
Two foliar endophytes isolated from Eastern white pine tissues were provided to us by our collaborators at J.D. Irving in Canada. These two fungal isolates, *Lophodermium nitens* and *Xylaria ellisii* have exhibited antifungal activity *in vitro. Lophodermium nitens* produces a compound known

as pyrenophorol which has inhibited the growth of *Cronartium ribicola* in lab experiments (Sumarah et al., 2015). *Xylaria ellisii* produces griseofulvin, another compound with demonstrated antifungal activity against plant pathogens (Park et al., 2005). Thus, we employed three seed lots varying in resistance to WPBR, and seven endophyte treatments.

HYPOTHESES or OBJECTIVES: The objective of this project is to compare WPBR resistance of selected WWP seed sources that are either inoculated, or not inoculated with promising endophytes. After inoculation with endophytes and then with rust in September 2018 seedlings were to be evaluated for the first time for rust symptoms and signs in 2019 in the CDA Nursery. However, it was apparent in 2019 that the 2018 rust inoculation had failed; susceptible, endophyte-free controls should have had signs and symptoms at the very least but they did not. All endophyte treatments of the susceptible seed lot were re-inoculated in the field with rust in September 2019, and we will evaluate them in the 2020 growing season in the nursery and score them for WPBR resistance traits so more results are still to come.

METHODS: Each individual treatment consisted of 196 seeds planted in individual cells. Each seed lot received each endophytic treatment. In addition, one set of 196 received no treatment to serve as a control. This treatment, like all others, was still be exposed to the white pine blister rust. Therefore, there was a total of 21 (3 x 7) unique treatments, each with 196 seeds. Artificial inoculations with WPBR followed procedures used at the USDA Forest Service Coeur d'Alene Nursery (Mahalovich 2010) but these failed due to a scheduling issue where seedlings were kept in the dark.

MAJOR FINDINGS: Endophyte #2 (*Streptomyces*) had a significant positive effect in two of the seed lots on emergence of seedlings. After exposure to the endophytes for 24 hours, the seeds had been sown. Control seeds received no endophyte treatment. Percent emergence was then recorded approximately three months later.

DELIVERABLES: We are about to submit the following manuscript that details the reasons for the inclusion of white pine seed endophytes as treatments against blister rust:
Busby, P., Marlin, M., Barge, E., Heitmann, S., Ridout, M., and G. Newcombe. 2020. **Seeds are home to taxa of bacteria, mostly *Bacillus*, and fungi, mostly Pleosporales, Hypocreales or Eurotiales, that are likely to be strong antagonists of pathogens of plants.** Proceedings of the Royal Society B: Biological Sciences. Since there still could be WPBR results there could be another manuscript.

References: Hoff, R.J. 1986. Inheritance of the bark reaction resistance mechanism in *Pinus monticola* infected by *Cronartium ribicola*. USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, UT, Research Note INT-361, 8 p.
Hoff, R.J. & G.I. McDonald. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: short shoot fungicidal reaction. *Canadian Journal of Botany* 49: 1235-1239.
McMullin, D.R., Nguyen, H.D.T., Daly, G.J., Menard, B.S., Miller, J.D. 2018. Detection of foliar endophytes and their metabolites in *Picea* and *Pinus* seedling needles. *Fungal Ecol.* 31: 1-8.
Newcombe, G., Harding, A., Ridout, M. and Busby, P., 2018. A hypothetical bottleneck in the plant microbiome. Frontiers in microbiology, 9, p.1645.
Richardson, S.N., Walker, A.K., Nsiama, T.K., McFarlane, J., Sumarah, M.W., Ibrahim, A., Miller, J.D. 2014. Griseofulvin-producing endophytes of *Pinus strobus* and *Vaccinium angustifolium*: evidence for a conifer-understory species endophyte ecology. *Fungal Ecol.* 11: 107-113.
Sumarah, M.W., Walker, A.K., Seifert, K.A., Todorov, A. & Miller, J.D., 2015. Screening of fungal endophytes isolated from eastern white pine needles. In *The Formation, Structure and Activity of Phytochemicals* (pp. 195-206). Springer, Cham.
Watve, M.G., Tickoo, R., Jog, M.M., & Bhole, B.D. 2001. How many antibiotics are produced by the genus *Streptomyces*? *Arch Microbiol* 176: 386-390.

MEMBER COMPANY BENEFITS: Endophyte #2 (*Streptomyces*) has positive effects on emergence of seedlings.