**PROJECT ID:** CAFS.23.XX

**PROJECT TITLE:** Enhancing Resistance to Fungal Pathogens in Commercial Tree Species

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| **PROJECT DESCRIPTION:** Endophytes are non-pathogenic microsymbionts, bacterial and fungal, that reside within all plant-associated tissues (Verma et al., 2017). Seeds have been shown to be an exclusionary niche for microbes resulting in a promising source for strong defensive endophytes, often in the genus *Bacillus*, which is well documented as having strong antagonists of pathogens and that produce antimicrobial compounds (Walker et al., 1988; Dong et al., 2004; Chaurasia et al., 2005; Khalaf and Raizada, 2018; Shahzad et al., 2018). There are theoretical (Newcombe et al., 2018) and mechanism-based (Raghavendra et al., 2013) reasons to think that it will be productive to assay species of *Bacillus* within plant-pathogen systems.  During a CAFS-NSF funded internship in 2022, a study was conducted testing endophytic treatments on susceptible seed of three tree species: western white pine (*Pinus monticola*; WWP), Port-Orford-cedar (*Chamaecyparis lawsoniana*;POC), and Hawaiian koa (*Acacia koa*) all commercially valuable species and of concern for restoration efforts due to devastation caused by pathogens. Three endophytic treatments were put to the test against the respective pathogen system (i.e., white pine blister rust - *Cronartium ribicola*; cedar root disease - *Phytophthora lateralis*; koa vascular wilt *Fusarium oxysporum* f. sp*. koae*); in the case of WWP two *Bacillus* treatments stood out as having a significant reduction in disease severity.  This led us to propose a new study in which we retest the repeatability of the significant findings, but we are also proposing to expand the project by including commercially planted ‘resistant’ varieties (e.g., WWP Bingham F2 lot). WWP Bingham F2 lot has demonstrated between 70-80% survival in some stands and about 33% or less in other stands due to white pine blister rust (Kearns, Brennan, and Schwandt, 2012). The overall goal of this proposed study will be to enhance survival and reduce disease severity in ‘resistant’ and susceptible varieties to improve out-planting success as seedlings are still often the preferred method to stand establishment over natural regeneration. |
| **HYPOTHESES and/or OBJECTIVES:** The objective of this project is to test the hypothesis that seed endophytes can enhance survival and reduce disease severity in susceptible and commercial ‘resistant’ varieties of seedlings against virulent strains of devastating pathogens:   1. *Acacia koa* against *Fusarium oxysporum f. sp. koae* 2. *Pinus monticola* against *Cronartium ribicola*. 3. *Chamaecyparis lawsoniana* against *Phytophthora lateralis*. |
| **METHODS:** Bacteria endophyte treatments (*Bacillus spp.*) will be grown out and provided by the University of Idaho in Moscow, Idaho. Abigail Ferson-Mitchell will be responsible for preparation and inoculation of all endophytic treatments. Seeds will be inoculated with the endophytic treatment prior to sowing and again prior to the pathogen inoculations.  The seed lots for western white pine and Port-Orford-cedar, sowing material, and greenhouse space for growing seedlings will be conducted at and supplied by the USDA- Forest Service, Dorena Genetic Research Center (DGRC) in Dorena, Oregon. Seed from Bingham F2 bulklot will be the ‘resistant’ family for western white pine and a quantitative gene resistant lot will be used for Port-Orford-cedar, in addition to repeating the susceptible families from the previous year. Pathogen inoculum for white pine blister rust (*Cronartium ribicola*) will be supplied by the USDA – Forest Service in the form of infected *Ribes spp.* leaves collected in the field prior to inoculation. Western white pine will be inoculated at DGRC, and post-rust inoculation assessment will occur at the facility every three months by Abigail Ferson-Mitchell. Data will be taken in the form of needle spot severity, canker severity, and mortality.  Port-Orford-cedar seedlings will be transferred to Oregon State University (OSU) and grown in their greenhouse space through the duration of the pathogen trial and data collection. Pathogen inoculum of cedar root disease (*Phytophthora lateralis*) will be supplied by OSU, Jared LeBouldes’s lab will conduct the pathogen inoculation with the assistance of Abigail Ferson-Mitchell. Data post-root disease inoculation and will be conducted once a month to run a time to event analysis.  The Hawaii Agricultural Research Center (HARC) in Kailua, Hawaii, has supplied the University of Idaho with nine strains of the pathogen (*Fusarium oxysporum f. sp. koae*) through a USDA approved permit. HARC will also supply the seeds for the study and the greenhouse trials will be performed at the University of Idaho. Koa seeds will be clipped, soaked in an endophytic bacterial spore suspension prior to sowing. After one-week seedlings will be transplanted into soil inoculated with the wilt pathogen. The trial will run for 100 days, and mortality counts are taken daily. A time to event analysis will be run on the data to compare the survival of the treatment groups to the control. |
| **PROJECT TIMELINE:**   * Summer 2023 – Inoculate western white pine and Port-Orford-cedar seed coming out of stratification with endophyte treatments and sow seeds. Take nine-month data from 2022 inoculation. Inoculate koa seed with endophytes and sow into pathogen inoculated soil; measure mortality for 100 days. * Fall 2023 – Inoculate western white pine with blister rust; take one year data from 2022 inoculation. Analyze data from 2022 inoculation: prepare manuscript. * Winter 2023 – Take data on 2023 western white pine inoculation; prepare Port-Orford-cedar seedlings to transfer to Oregon State University. Final data on first round koa mortality. * Spring 2024 – Inoculate Port-Orford-cedar at OSU with cedar root disease. Begin additional round of koa wilt assay for 100 days. * Summer 2024- Take data on POC, WWP, and final koa data. Analyze and prepare manuscript for koa results. * Fall 2024 – One year data on WWP and nine-month data on POC. Begin analysis and preparation of manuscripts. * Winter (November) 2024- Finish data analysis, submit CAFS final report, and publish manuscripts. |
| **EXPECTED DELIVERABLES – ONE YEAR:** A publication, dissertation, and a new protocol developed for screening bacterial endophytes for beneficial effects against pathogens is anticipated. |
| **EXPECTED DELIVERABLES – LONG-TERM:** A minimum of three publications in addition to the dissertation is anticipated from this research. |
| **POTENTIAL MEMBER COMPANY BENEFITS:** Results could be used to improve survival of out-planted resistant varieties in different ecoregions, reducing the cost and effort for reforestation following a failed plantation in areas impacted by low-medium-high pathogen loads. The results may also be used to improve survival of genetic families that lack genes for resistance but have other desirable traits for commercial use (e.g., hardiness, timber quality, fast growth). Finally, the results may also be used to inform tree improvement programs as endophytes isolated and screened for this research can naturally occur in seed through maternal transfer, this may explain some variation in trial results observed in these programs. |
| **NEXT YEAR’S PROJECT BUDGET – NSF/CAFS PORTION:** Funds are requested through the end of CAFS - Phase III (November 2024), no request for additional NSF-CAFS funding is projected for next year. |
| **NEXT YEAR’S PROJECT BUDGET - OTHER SOURCES, INCLUDING SITE-SPECIFIC:** Additional funding will come through a USDA NIFA funded joint program to PI George Newcombe, to fund REEU undergraduate students to assist in research on utilizing seed endophytes to enhance seedling success and survival. |
| **REFERENCES:**   * Chaurasia, B., Pandey, A., Palni, L.M.S., Trivedi, P., Kumar, B., Colvin, N., 2005. Diffusible and volatile compounds produced by an antagonistic Bacillus subtilis strain cause structural deformations in pathogenic fungi in vitro. Microbiol Res 160, 75–81. * Dong, Y.-H., Zhang, X.-F., Xu, J.-L., Zhang, L.-H., 2004. Insecticidal Bacillus thuringiensis silences Erwinia carotovora virulence by a new form of microbial antagonism, Signal Interference. Appl Environ Microbiol 70, 954–960. * Kearns, H.S.J., Ferguson, B.A., Schwandt, J.W., 2012.Performance of rust-resistant western white pine in operational plantations in northern Idaho: 1995-2006. Forest Health Protection, Report 12-03. * Khalaf, E.M., Raizada, M.N., 2018. Bacterial seed endophytes of domesticated cucurbits antagonize fungal and oomycete pathogens including powdery mildew. Front. Microbiol. 9. * Newcombe, G., Harding, A., Ridout, M., Busby, P.E., 2018. A hypothetical bottleneck in the plant microbiome. Front. Microbiol. 9. * Raghavendra, A.K.H., Newcombe, G., Shipunov, A., Baynes, M., Tank, D., 2013. Exclusionary interactions among diverse fungi infecting developing seeds of Centaurea stoebe. FEMS Microbiology Ecology 84, 143–153. * Shahzad, R., Khan, A.L., Bilal, S., Asaf, S., Lee, I.-J., 2018. What is there in seeds? vertically transmitted endophytic resources for sustainable improvement in plant growth. front. Plant Sci. 9. * Verma, S.K., Kingsley, K., Irizarry, I., Bergen, M., Kharwar, R.N., White, J.F., 2017. Seed-vectored endophytic bacteria modulate development of rice seedlings. Journal of Applied Microbiology 122, 1680–1691. * Walker, R., Powell, A.A., Seddon, B., 1998. Bacillus isolates from the spermosphere of peas and dwarf French beans with antifungal activity against Botrytis cinerea and Pythium species. J Appl Microbiol 84, 791–801. |