Physiological and molecular responses of lodgepole and jack pine trees under environmental stress to the blue stain fungus, *Grosmannia clavigera*

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Background

Conifers exhibit both constitutive and induced defense mechanisms in response to attack by pests and pathogens, such as mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and their fungal associates. However, this defense can be modulated by environment, where abiotic stress may reduce the species ability to respond to biotic stress. In this study, we examined the relative influence of water limitation on constitutive and induced defenses of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *douglar*), and the naive host jack pine (*P. banksiana* Lamb.) to inoculation with the mountain pine beetle fungal associate *Grosmannia clavigera*. Molecular and biochemical assays evaluated inducible defense responses of jack and lodgepole pine, in response to inoculation with *G. clavigera* at 1, 7, and 28 days post-inoculation (dpi).

Our objective was to evaluate the influence of water deficit in tree responses to *G. clavigera*, and identify differences in constitutive and induced defenses between lodgepole and jack pine.

Experiments

**Fig. 1.** Experimental set up for lodgepole (A) and jack mature pines (B), and seedlings (C). Water deficit was induced by placing woven laminated polyethylene tarps.

**Seedlings:** Second year seedlings of lodgepole, and jack pine were grown in Sunshine growing media 4, and maintained under controlled conditions as follows: 19°C (day/night), 20-35% RH, and photoperiod 15h day light (200 μmol PAR) and 9h night. A total of 4 independent experiments with 8 biological replicates per experiment were sampled across treatment combinations.

**Mature trees:** Natural stands of jack and planted lodgepole pines trees located in Smoky lake Alberta were used. Ten trees of similar diameter breast height (dbh) were randomly selected for each treatment combination. A two by three factorial design with two treatment factors, water level and fungus inoculation with *G. clavigera* was performed.

Physiological, biochemical & transcriptional analyses:

**Seeding collection:** 1, 7, 14, 28, 35, 56 and 68 days post inoculation (dpi)

**Mature tree collection:** 20, 28, 50 and 70 dpi

**Fig. 2.** (A) Inoculation and, (B) Lesion length of mature trees after five weeks of inoculation. (C) Lesion developed in pine seedlings after 28 days post inoculation.

Acknowledgements

We thank Leonard Barnhardt for providing the experimental field site at Smoky lake (AB), and also for his valuable help during the experiment. We also want to thank Dr. Walel E1 Elsayed and Charles Copeland for their help with this project, and Marta Musken for her help with the microscopy analyses.

Results & Discussion

**Fig. 3.** Lesion length is generally associated with fungal growth and/or the response to the fungi. Under well-watered conditions lesions were longer in lodgepole pine relative to jack pine, and its development is slowed under water deficit. However, after a longer exposure to the fungi, water deficit trees developed longer lesions, which could be a higher fungal development and a delayed tree response.

**Fig. 5.** Anatomical changes of inoculated pine seedling under water deficit conditions. Inoculation resulted in swollen polyphenolic parenchyma (PP) cells in lodgepole pine, and development of additional phenolic bands (PB) in both species, indicating an induced defense against *G. clavigera*.

**Fig. 7.** Jasmonic acid (JA) and salicylic acid (SA) are important for defense signaling in response to insects and pathogen attack. Fungal inoculation results in increased JA accumulation in both, jack and lodgepole pine. SA increased significantly in jack pine under fungal inoculation in water deficit treatment, probably as an active defense mechanism.

Conclusions

Fine-scale differentiation among functional categories of differentially expressed genes point towards species-specific strategies associated with defense. These data suggest that water limitation modulates defense response, and abiotic and biotic factors may interact to influence the transition of MPB and associated pathogens from lodgepole pine to jack pine.