University of Victoria | Graduate Studies

Notice of the Final Oral Examination for the Degree of Doctor of Philosophy

of

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“Resistance mechanisms to *Didymascella thujina* (Durand) Maire in *Thuja plicata* Donn ex D. Don, *Thuja standishii* (Gord.) Carr. and *Thuja standishii* × *plicata*”

Department of Biology

Wednesday, August 29, 2018
10:00 A.M.
Clearihue Building
Room B007

Supervisory Committee:
Dr. Barbara Hawkins, Department of Biology, University of Victoria (Co-Supervisor)
Dr. John Russell, Department of Biology, UVic (Co-Supervisor)
Dr. Peter Constabel, Department of Biology, UVic (Member)
Dr. Olaf Niemann, Department of Geography, UVic (Outside Member)
Dr. Jim Mattson, Department of Biological Sciences, Simon Fraser University (Additional Member)

External Examiner:
Dr. Nadir Erbilgin, Department of Renewable Resources, University of Alberta

Chair of Oral Examination:
Dr. David Berg, Department of Chemistry, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Plants and microorganisms interact with each other constantly, with some interactions being mutually beneficial and others being detrimental to the plants. Interactions that cause plant diseases are studied in the field of plant pathology. The features of the organisms involved in such interactions will determine the characteristics of individual pathosystems. Plants respond readily to pathogen attacks, regardless of the pathosystem, furthermore, variation in the resistance to pathogens within species is common and well documented in many plants. The variability in pathogen resistance is at the core of genetic improvement programs for disease resistance. True resistance to pathogens in plants is a genetically determined and complex trait that can involve both constitutive and induced mechanisms at different levels of organization. The complexity of this phenomenon makes the study of compatible plant – pathogen interactions challenging, and typically, disease resistance studies focus on specific aspects of a pathosystem, such as field resistance, anatomical or physiological features of resistant plants, or molecular mechanisms of resistance.

The *Thuja* sp. - *Didymascella thujina* (E.J. Durand) Maire interaction is an important pathosystem in western North America, which has been studied for more than five decades. Western redcedar (*Thuja plicata* Donn ex D. Don) is very susceptible to cedar leaf blight (*D. thujina*), a biotroph that affects the tree at all stages, although seedlings are the most sensitive to the disease. The characteristics of the *Thuja* sp. - *D. thujina* interaction, the wealth of information on the pathosystem and the excellent *Thuja* sp. genetic resources available from the British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development make this interaction an ideal system to advance the study of disease resistance mechanisms in conifers. This Doctoral project presents a comprehensive investigation of the constitutive and induced resistance mechanisms against *D. thujina* in *T. plicata*, *Thuja standishii* (Gord.) Carr. and a *Thuja standishii* × *plicata* hybrid at the phenotypic and gene expression levels, undertaken with the objective of exploring the resistance mechanisms against the biotroph in these conifers. The project also aimed to establish base knowledge for the future development of markers for marker assisted breeding of *T. plicata*.

The investigations included a combination of histological, chemical and next generation sequencing (NGS) methodologies. NGS data were analyzed, in addition to the traditional clustering analyses, with cutting edge machine learning methods, including grade of membership analysis, dynamic topic modelling and stability selection analysis. The studies were progressively more controlled to narrow the focus on the resistance mechanisms to *D. thujina* in *Thuja* sp. Histological characteristics related to *D. thujina* resistance in *Thuja* sp. were studied first, along with the relationship between climate of origin and disease resistance. The

virulence of *D. thujina* was also documented early in this project. Chemical and gene expression constitutive and induced responses to *D. thujina* infection in *T. plicata* seedlings were studied next. *T. plicata* clonal lines were then comprehensively studied to shed light on the mechanisms behind known physiologically determined resistance. A holistic investigation of the resistance mechanisms to *D. thujina* in *T. standishii*, *T. plicata* and a *T. standishii* × *plicata* hybrid explored the possibility of a gene-for-gene resistance model.

Thirty-five *T. plicata* families were screened during the four field seasons carried out between 2012 and 2015, totalling more than 1,400 seedlings scored for *D. thujina* severity. Thirteen of those families were used in the five studies run during the program, along with two *T. plicata* seedling lines self-pollinated for five generations and three *T. plicata* clonal lines. One *T. standishii* clonal line, and one *T. standishii* × *plicata* clone were also investigated during the program. A total of 16 histological and anatomical characteristics were studied in more than 750 samples, and more than 270 foliar samples were analyzed for 60 chemical and nutritional compounds. Almost one million transcriptomic sequences in four individually assembled reference transcriptomes were examined during the program.

The results of the project support the variability in the resistance to *D. thujina* in *T. plicata*, as well as the higher resistance to the disease in plants originating from cooler and wetter environments. The data collected also depicted the existence of age-related resistance in *T. plicata*, and confirmed the full resistance to the disease in *T. standishii*. Western redcedar plants resistant and susceptible to *D. thujina* showed constitutive differences at the phenotypic and gene expression levels. Resistant *T. plicata* seedlings had thicker cuticles, constitutively higher concentrations of sabinene, *α*-thujene, and higher levels of expression of NBS-LRR disease resistance proteins. Resistant clones of *T. plicata* and *T. standishii* had higher expression levels of bark storage proteins and of dirigent proteins. *Thuja* sp. plants from all ages, species and resistance classes studied that were infected with *D. thujina* showed the accumulation of aluminum in the foliage, and increased levels of sequences involved in cell wall reinforcement. Additional responses to *D. thujina* infection in *T. plicata* seedlings included the downregulation of some secondary metabolic pathways, whereas pathogenesis-related proteins were upregulated in clonal lines of *T. plicata*. The comprehensive approach used here to study the *Thuja* sp. - *D. thujina* pathosystem can be applied to other compatible plant-pathogen interactions.