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

of

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BSc Hons. (University of Victoria, 2005)

“Functional Analysis of Proteins in the Conifer Ovular Secretion”

Department of Biology

Monday, August 17, 2020
9:00 A.M.
Conducted Remotely

Supervisory Committee:
Dr. Patrick von Aderkas, Department of Biology, University of Victoria (Supervisor)
Dr. Barbara Hawkins, Department of Biology, UVic (Member)
Dr. Ben Koop, Department of Biology, UVic (Member)
Dr. Tom Fyles, Department of Chemistry, UVic (Outside Member)

External Examiner:
Prof. Art Davis, Department of Biology, University of Saskatchewan

Chair of Oral Examination:
Dr. Sylvia Pantaleo, Department of Curriculum and Instruction, UVic

Dr. Stephen Evans, Acting Dean, Faculty of Graduate Studies
Abstract
Almost all conifer ovules produce a liquid secretion as part of reproduction. This secretion, termed an ovular secretion, is produced during ovule receptivity and is involved in pollen capture and transport. Historically, examinations of the ovular secretion have focused on how they are part of pollination mechanisms. As a result, the chemical composition of the ovular secretion has not been examined systematically. Investigations into the constituents of the ovular secretion were limited to analyses for simple water soluble compounds such as sugars, minerals, amino acids and organic acids. More recently, the protein component of the secretion has been investigated using mass spectrometry-based proteomics. Proteins involved in processes such as carbohydrate modification, proteolysis, and defence have been identified in conifer ovular secretions. This biochemical complexity suggests a broader view of the function of the ovular secretion is warranted. However, protein identifications only provide putative information on function. Functional characterization of these proteins is needed in order to fully understand how they contribute to ovular secretion function. The research outlined in this dissertation describes the first functional characterizations of proteins found in conifer ovular secretions. Three proteins – invertase, chitinase, and thaumatin-like protein – were characterized in the ovular secretions of Douglas-fir (*Pseudotsuga menziesii*) and hybrid yew (*Taxus × media*).

The Douglas-fir ovular secretion is capable of converting sucrose to glucose and fructose, confirming that invertases present in the secretion are functional. The invertase activity was maximal at pH 4.0. Activity was 77% of maximal at pH 4.5, the physiological pH. This indicates that post-secretory hydrolysis of sucrose occurs *in situ* in the Douglas-fir ovular secretion. Invertases in the ovular secretion are likely involved in controlling the movement of carbohydrates to developing pollen and could facilitate pollen selection.

Chitinases present in the Douglas-fir ovular secretion are functional at physiological conditions. All three modes of chitinolytic activity, i.e. endochitinase, chitobiosidase and β-N-acetylglucosaminidase, were detected at physiological pH. β-N-acetylglucosaminidase activity was 80% of maximal at physiological pH. Chitinases are pathogenesis-related proteins capable of hydrolysing chitin in fungal cell walls. These results suggest the ovular secretion is capable of defending the ovule against infection by phytopathogens.
Thaumatin-like protein was immunolocalized to the cell wall and amyoplasts in Douglas-fir and yew nucellar tissue in a pattern consistent with a defensive role. It was also localized to the cell wall of fungal spores and germinating hyphae that were present in the micropyle of a yew ovule. These results provide additional evidence for an antifungal role for the ovular secretion.

Functioning enzymes involved in pollen-ovule interactions and ovule defence are present in the conifer ovular secretion. The ovular secretion has functions beyond pollen capture. A revised functional model for the conifer ovular secretion is proposed.