Full name(s) of VIU faculty involved in the project and their Department(s): Jasmine Janes, Biology; Caroline Josefsson, Biology

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Award: Explore Grant

Number of undergraduate student(s) involved: 1 paid

Number of graduate student(s) involved: NA

Number of community partner(s) involved: NA

Abstract for the project:
Mycorrhizae are ecologically important symbiotic relationships between plants and fungi. The significance of such relationships is greatest within the orchid family. Orchids exhibit complete dependence on mycorrhizae to germinate, as orchid seeds lack the nutritional reserves present in other plant groups. Thus, the distribution of orchid species is often limited by the distribution of suitable fungal partners. Epipactis helleborine is a terrestrial orchid that was introduced into North America from Europe and northern Africa. Since its introduction, the species has become widespread, suggesting that E. helleborine associates with a wide variety of fungal partners in this new environment. Studies in its native range have confirmed that E. helleborine is a ‘generalist’ in terms of mycorrhizae, but no studies have been conducted in North America to verify its degree of fungal specificity. Using fungal isolation methods and DNA sequencing technology we will characterize and compare the fungal specificity exhibited by E. helleborine in central Vancouver Island. These data are useful for understanding: 1) mycorrhizal associations and fungal species diversity, 2) the extent to which E. helleborine may continue to spread, and 3) the evolutionary biology of invasive species once they establish in new environments.

Specificity of mycorrhizal associations in the introduced orchid *Epipactis helleborine*

Background

Mycorrhizae are an important, yet often understudied, component in any ecosystem (Tedersoo 2017). They represent the fungal symbiotic relationship with plants in which both parties receive benefit. The fungus typically receives additional carbohydrates from the plant, while the plant gains access to more mineral nutrients through the fungal network (Albornoz *et al.* 2016). Thus, many plant groups evolve such relationships during some point in their life history. However, the Orchidaceae are the only plant group known to exhibit complete dependence on mycorrhizae for germination (Esposito *et al.* 2016).

Orchid species possess seed that are devoid of nutrient reserves (Esposito *et al.* 2016). Consequently, the seed is microscopic and readily dispersed long distances. To germinate the seed must encounter a suitable symbiotic mycorrhiza so that it can gain nutrients to fuel growth (Waud *et al.* 2017). Thus, the distribution of orchid taxa is dependent on dispersal mechanisms and fungal distribution. These relationships can be complicated further by the fact that some orchid species exhibit species-level (one orchid, one fungus) or taxon-level specificity (one orchid, multiple fungi within a form-genus), or non-specificity, with fungal partners (Warcup 1991).

Epipactis helleborine is a terrestrial orchid native to Europe and North Africa that was introduced to North America (Suetsugu *et al.* 2017). Since its introduction in 1879 the species has spread widely, occurring in a variety of habitats from wetlands to deciduous forest (Argue 2012). Previous research within Europe suggests that *E. helleborine* is capable of associating with a wide range of mycorrhiza, which likely facilitated its spread throughout North America (Tesitelova *et al.* 2012, Jacquemyn *et al.* 2016). A review of the literature indicates that investigations of *E. helleborine* mycorrhizal specificity are absent within North America. This study aims to characterize the mycorrhizal associations of *E. helleborine* on Vancouver Island.

Methods

Site selection

Personal observations of *E. helleborine* have been made at Milner Gardens, Linley Valley and the Cassidy area of south Nanaimo. Collection efforts will focus in these areas but opportunistic collections elsewhere will be made when possible.

Collection of fungal samples

Samples will be collected during peak flowering (June-August) to ensure that correct species identification can be made. Collection methods will follow those of Janes (2009). Briefly, soil will be removed from around the plant to expose the tubers. A small cutting of the tuber will be collected and placed in sterile water. A minimum of five individuals will be samples from each site depending on population numbers. Photographs of the individual plants will be taken for reference purposes.

Isolation and identification of fungal samples
Orchid tuber tissues will be processed according to Janes (2009). The tissue will be rinsed in sterile water before being macerated under laminar flow using aseptic technique. Fungal pelotons will be extracted using microcapillary tubes and plated onto fungal isolation media (FIM). A minimum of five plates per orchid individual, each containing three pelotons, will be prepared. High numbers of replicate plates per individual will be necessary to account for contamination and potentially high numbers of different fungal isolates. Plates will be observed for fungal growth and sections of hyphae will be re-plated to obtain pure cultures.

Visual assessments of fungal characteristics will be recorded. However, fungal species can be exceptionally difficult to identify if they lack sexual features. Thus, Sanger sequencing of the internal transcribed spacer region (ITS) will be performed on pure cultures to confirm fungal identifications (Wright *et al.* 2010). Sequences will be entered into GenBank.

Data analysis
Sanger sequences will be aligned using Geneious. Evolutionary relationships among fungal isolates will be reconstructed using the MEGA add-in for Geneious. Diversity of fungal types within and between sites will be assessed and compared using one-way ANOVA.

Activity	**June-Aug**	**Sept-Nov**	**Dec-Feb**	**Mar-May**
Sample collection	X			
Sample processing	X	X		
Sequencing		X	X	
Data analysis			X	X
Expenditure of funds	X			

Outputs/outcomes
This project will provide insight into: 1) how *E. helleborine* is able to colonize new areas so rapidly and successfully, 2) the degree of mycorrhizal specificity exhibited by *E. helleborine* outside of its natural range, and 3) how widespread suitable mycorrhizae are on the Vancouver Island landscape. This project will also provide substantial training for an undergraduate student in the areas of plant and fungal identification, molecular ecology, phylogenetics, aseptic technique and data analysis.

Plans to mobilize/commercialize knowledge/outputs
Findings from this project will be communicated through the VIU Research and Creative Activity Symposium and VIU's Research Week. In addition, the student will be encouraged to present the work at the Pacific Ecology and Evolution Conference (PEEC) in Bamfield. We will also pursue a publication in *Mycorrhiza*.

References

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Item	Quantity	Justification	Total cost
Salary	1	As of June 1st 2019, the minimum wage for BC is $13.85/hr. The student will be employed over the summer to begin fieldwork. To ensure that a student is attracted and motivated to perform the work, it is necessary to pay at least minimum wage. The budgeted funds will be paid to the student, providing approximately four weeks of salary. The BIOL491 aspect of the project will commence in Fall 2019, comprising literature reviews, statistical analysis and thesis writing. This component will be unpaid.	$2,000
TOTAL			**$2,000**

Project team – roles & responsibilities

Dr Jasmine Janes – As **co-principal investigator** I will provide expertise in experimental design, statistical analysis, plant identification and fungal culture.

Dr Caroline Josefsson (co-principal investigator) – provides expertise in sequencing, plant identification and fungal culture.

BIOL491 student – An exemplary student has been recruited to undertake this work as part of their BIOL491 project. This student (Cassandra Twiname) has taken several upper level courses in preparation for this type of research, including: plant ecology, terrestrial ecosystems, botany and microbiology.