

Getting to the root of the problem with terrestrial orchid conservation: identifying the fungal symbionts of ram's-head lady slipper (*Cypripedium arietinum*) in Nova Scotia

* **Katie King**¹, Gavin Kernaghan², Allison K. Walker¹

¹ Department of Biology, Acadia University, Wolfville, Nova Scotia

² Department of Biology, Mount Saint Vincent University, Halifax, Nova Scotia

Cypripedium arietinum is an endangered orchid found in Eastern North America. It relies on symbiotic fungi for seed germination and nutrient transfer, and conservation efforts are hampered as researchers of this species have yet to identify the fungal partners involved. The Nova Scotian population is disjunct and found in a different habitat type than most other known populations, factors which may influence the fungi that associate with *C. arietinum*. Root and soil samples were collected under permit from each of seven sites in the spring and early summer of 2024, and we have begun to isolate and identify fungi present in mature plants. Roots were examined for specific fungal structures found in orchid mycorrhizae called pelotons and dissected to isolate fungi onto selective media. Fungal DNA was also extracted from root tissue for PacBio next generation sequencing. Seed collections are planned for *ex situ* seed baiting trials for fungi, and the fungi found in the tissues of any germinated protocorms will be similarly identified using culture and molecular techniques. We have isolated fungi from root samples from each of seven sites including known orchid symbionts from the genus *Tulasnella* and ectomycorrhizal species from the genus *Tomentellopsis*. We aim to fill this crucial knowledge gap regarding *C. arietinum* and identify fungi that play essential roles in the germination and growth of this endangered plant, which has relevance for propagation and conservation.

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