

## **Axenic Seed Culture of *Cypripedium arietinum***

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*Cypripedium arietinum*, the ram's head lady's slipper, is a critically endangered plant in northern New England and one of the most challenging lady's slippers to raise in sterile culture. Our main goal is to develop a technique for faster germination in axenic seed culture by manipulation of standard media used to grow other species of this genus. To support this goal, we studied the histological structures of the flower for a better understanding of the plant's reproduction. In the present study, we evaluated the germination rate in response to increasing amounts of coconut water, a known nutrient, from 10% (standard concentration) to 15 and 20% in the growth medium as well as the impact of light and dark environments. We also started collecting plant samples to analyze the plant's reproductive organs. By 6 weeks post seed inoculation, similar germination rates were observed between the groups that received 10 and 20% of coconut milk while for unknown reasons no germination was observed in the group exposed that received 15% coconut milk. None of the seeds exposed to light germinated. These data suggested that: 1) exposure to light could prevent or at least severely delay germination and, 2) changes in coconut milk concentration did not affect germination rate. Future experiments will continue this work by further varying the concentration of coconut milk in the growth medium. Finally, using formalin-fixed paraffin embedded samples and standard H&E (hematoxylin and eosin) staining methods, we produced numerous sections with undistorted cells and few obvious artifacts. Examination of a mature unfertilized ovary have so far revealed similar histological organization to other *Cypripedium* species, with clearly defined ovaries and seedpod ridges with vascular tissues immediately internal to the ridges.