

## ***Axenic In Vitro Culture of Cypripedium Seeds***

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*Cypripedium reginae*, *Cypripedium arietinum*, and *Cypripedium parviflorum* are endangered throughout New England, primarily due to habitat degradation/loss and illegal collection. If these plants were readily available through garden clubs and commercial greenhouse concerns, it would provide a pragmatic solution to population decline. An ongoing collaboration with the New Hampshire Orchid Society will help provide healthy seedlings to conservationists in northern New England. The use of axenic seed culture to produce thousands of *Cypripedium* plants and the development of a method of ensuring survival in soil is central to this restoration effort. Axenic culture involves surface disinfestation of seeds followed by inoculation into culture tubes with 20 mls of Murashige and Skoog basal salts media, solidified with 0.7% agar and fortified with coconut milk and sucrose. Approximately 50% of *Cyp. reginae* seeds germinated, yielding 5088 seedlings in culture. Of these seedlings, 2304 have not yet developed prominent roots or shoots while 2784 have generated well-developed roots and shoots. Transferring seedlings to fresh culture medium was hypothesized to speed growth. After approximately 1 year in culture, the average non-transferred seedling weighed 0.06 grams, whereas the average transferred seedling weighed 0.27 grams indicating a 450% increase in the sizes of transferred seedlings. Optimizing germination rates in *Cyp. parviflorum* and *Cyp. arietinum* by manipulation of sucrose and coconut milk supplementation should provide a method for increased robustness and viability of seeds. This could provide a partial solution to population decline of these two endangered terrestrial orchids.