

Protocols for Propagation of *Cypripedium* Species to Prevent Further Population Decline in Northern New England

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Terrestrial orchid populations of the *Cypripedium* genus are declining primarily because of habitat loss and degradation as well as illegal collection. *Cypripedium arietinum*, *Cyp. parviflorum*, and *Cyp. reginae*, commonly called lady's slippers, are regionally endangered. Lady's slippers take about 10 years to develop their first flowers in the wild. In vitro sterile culture procedures can help speed up this process to about 3 years, but little scientific work has been done to support this as a means to help prevent population decline in the wild. We are developing techniques to propagate large numbers of these plants in sterile culture and ensure maximum survival after transplantation to soil and eventually to their natural habitat. Attempts to propagate sufficient numbers of *Cyp. parviflorum* and *Cyp. arietinum* were not successful, yielding less than 1% germination from over 1000 seeds in culture for each species. Modification of sterile culture media components has shown some improvement in germination of these two species. About 5,000 *Cypripedium reginae* seedlings were grown in sterile culture until well-developed roots and shoots formed. Further experiments were focused on *Cyp. reginae* and developing the most efficient means of vernalization (prolonged exposure to cold) that is believed to be necessary for the growth and development of this species in the wild. Seventy-six seedlings were placed in cold frames for an entire summer. Three seedlings grew small leaves and the remaining 73 seedlings were healthy but dormant. This is not surprising since vernalization is generally believed to be necessary for shoot growth in the wild. These plants in cold frames will be placed in the ground for the winter and monitored for shoots producing leaves in the spring. About 175 additional seedlings were refrigerated at 3°C, transferred to pots in green house conditions, and are being monitored for growth of roots and leaves.

