

Improved Propagation of the Showy Lady's Slipper to Support a Restoration Program

Katherine Duan and Alexander Kish - Crossroads Academy, Lyme NH
New Hampshire Academy of Science - 2016

The showy lady's slipper, *Cypripedium reginae*, is a prime subject for modeling a restoration study because of its long maturation period and sensitivity to changes in its environment. It is critically endangered because of habitat degradation as well as poor germination and survival in the wild. We conducted experiments to improve *in vitro* germination and to increase survival rates after vernalization and transplantation to soil in order to support a large-scale restoration of the species to New Hampshire. We hypothesize that under optimal conditions we can reduce the time it takes to reach a mature flowering plant from about 10 years in the wild to about 5 years in a controlled environment. We built a sterile hood and conducted a test of the hood's effectiveness and found it was fully operational to help in our efficient sterile culture of the thousands of seedlings we will need. A germination study of stored seeds showed that seeds can be stored at 5°C for up to 4 years and that seedpods can differ substantially in their seed viability. We also tested three methods of seedling vernalization at 5°C: storing in a mixture of peat moss and compost, storing as bare roots, and storing sterile agar cultures directly in the fridge without transferring to another medium. The results revealed that vernalizing the agar cultures without transferring was most effective, yielding a 100% survival rate. To improve transplantation efficiency, we created a novel hydroponic propagation system that circulated a nutrient solution through trays containing showy lady's slipper seedlings grown in axenic culture. This system produced sprouts in the first week, with seedlings reaching heights between 5 and 6 centimeters by the end of 5 weeks. This is a substantially more efficient method than growing in soil. To ensure maximum survival in the wild, over the past 3 years we have tested methods of transplantation into outside environments using four artificial fens. Of these, the oldest fen had about 18 healthy 4-year-old plants, with the largest about 25 centimeters tall. The largest of these plants are expected to flower next year, indicating we have reduced time to maturity by about 50%. Two of the other three fens had 18 and 12 healthy 2-year-old seedlings. The remaining fen had no success; most likely from the lack of proper watering and location in too dry an area.