Propagation of the Showy Lady's Slipper

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Abstract

The showy lady's slipper is critically endangered and a prime subject for modeling a restoration study. We conducted experiments to improve germination *in vitro*, 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 Class II 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 5ºC for up to 4 years and that seed pods can differ substantially in their seed viability. We tested three methods of vernalization: using a mixture of peat moss and compost, using the common bare root method, and placing sterile agar cultures directly in the fridge without transferring to another medium. The results revealed that soil storage was the least effective, bare root only marginally better, and storing the agar cultures without transferring was 100% effective. To improve transplantation efficiency, we created a novel hydroponic system that circulated 1/10th strength Murashige & Skoog nutrient solution through 4 propagation trays containing showy lady's slipper seedlings grown in axenic culture. Two of the trays contained Growstone® Hydro stones topped with a ½-inch layer of chopped Grodan® "rock wool". The other trays contained ¾-inch cubes of Grodan® "rock wool" topped with a ½-inch layer of chopped Grodan® "rock wool". Tests revealed that the system produced sprouts within the first week and, by the end of five weeks, seedlings reached heights between 5cm and 6cm. This is a substantially more efficient method than growing in soil. We have constructed four artificial fens in the past 3 years. Of these, the oldest fen had about 18 healthy 4-year-old plants, with the largest about 25 centimeters tall. Two of the other 3 fens have 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.

Introduction

The showy lady's slipper, *Cypredium reginae,* is a flagship of endangered species around the world. It is critically endangered in New Hampshire where its habitat is rare. In the wild, there is a less than 1% seed germination rate. Of those that germinate, less than 1% will reach maturity (Faletra et. al, 1997). Although we have improved the germination rate *in vitro* for the showy lady's slipper, the survival rates after vernalization and after transplanting to soil and are far below optimal. The purpose of this study is to maximize germination *in vitro* as well as to improve survival after vernalization and transplantation to soil. We hypothesize that under optimal controlled conditions, we can reduce the time it takes to reach a mature flowering stage from 8-10 years in the wild to about 4-5 years. We are also studying the histological properties of slippers to help in the ultimate goal of cloning a showy lady's slipper. We present a series of experiments that include: germination of seeds stored for up to 4 years, survival after vernalization under various conditions, transplantation to artificial fens, growth in a semi-automated soilless hydroponic system, and building and testing a Class II sterile hood.

Methods

Comparing Seed Viability Among 1, 2, 3, and 4 Year-Old Seeds

Seeds from mature, un-dehisced seedpods of *Cypripedium reginae* collected in late September to early October of 2011, 2012, 2013, and 2014 were used in this experiment. After the seedpods were harvested, they were allowed to dry for about a week. The seeds were stored at 5 ºC. Seeds were cultured and monitored for germination and development according to the procedure published by Sokolski et al, 1997 and Faletra et. al, 1997.

Building and Testing a Class II Sterile Hood

We designed and built a Class II sterile hood. We conducted a test of sterility during transfers of cultures in the hood at three fan speeds: medium, medium-high, and high. Each speed had 6 culture vessels. Two culture vessels were unopened and served as a control group.

Comparing 3 Methods of Vernalization

We tested two common methods and one new method of vernalization. We tested a standard medium of vernalization, which is a mixture of peat moss and compost. We tried both the standard medium and Vermont compost ®. Seedlings with prominent roots and shoots were layered in either compost and peat moss or Vermont compost ®. We also tried vernalizing seedlings using the bare-root method often used by commercial growers. The new method of vernalization that we tested was placing the agar cultures in the fridge without transferring the seedlings to a different medium. All seedlings were stored at 3-5ºC in a refrigerator.

Fen Construction

We have created 4 artificial fens in locations that received between six to eight hours of direct sunlight. We removed the top 12 inches of the soil and mixed half of the original soil with an equal amount of Vermont composted manure and peat moss. We added a handful of pelletized lime. We lined the hole with plastic and poked holes in the plastic, for the water to drain. We filled the hole with the planting mixture and planted the seedlings about an inch deep to make sure roots and shoots were not exposed. In the fall, we covered the fen with straw. Seedlings were monitored in the spring for new shoots.

Testing a novel hydroponic system for improved sprouting of vernalized seedlings

Four plastic 27 liter boxes measuring 15”Wx 21”Lx 5.5”H were used as propagation trays (Fig. 1). Each one had two ½-inch holes for the entrance of the solution and two larger ¾-inch holes for the exit of the solution. Each tray also had one ½-inch hole at the bottom of the tray nearest to the exit holes for a complete drain of the systems when desired. All of the trays were covered with black tape to keep out light and prevent any algae from growing. All the trays were placed on a custom platform that tilted the trays with a ¾-inch rise away from the entrance of the solution. The table also had two control panels (Fig. 2) that held all the valves to regulate the flow of the solution. Three 4-foot grow lights and three 2-foot grow lights were on timers to mimic normal seasonal light exposure. A 1/10th strength dilution of Murashige & Skoog nutrient solution circulated through the trays. Two trays were filled part way with Growstone® Hydro stones as a bottom layer with a ½-inch top layer of chopped Grodan® “rock wool”. The other two trays were filled part way with ¾-inch cubes of Grodan® “rock wool” as a bottom layer with a ½-inch top layer of chopped Grodan® “rock wool”. Pictures were taken of seedlings before planting in the upper rock wool layers and flagged for identification. The seedlings were monitored each

(Fig. 1) The complete hydroponic

(Fig. 2) One of the two control panels.

day for seedling height, changes in humidity, reservoir pH, and temperatures.

Results and Discussion

Comparing Seed Viability Among 1, 2, 3, and 4 Year-Old Seeds

After 167 days, seeds collected in 2011, 2012, 2013, and 2014 had a total germination percentage of 77.55%, 6.96%, 36.00%, and 98.57% respectively. As shown in Figure 3, there appears to be little correlation between the age of seeds and germination percentage, since seeds from 2011 had nearly the same germination rate as those of seeds from 2014. Seeds originally having poor seed viability may cause this. To answer this question, one might conduct an experiment where the same seed pod is used to inoculate seeds in 4 successive years. This will eliminate the major variable of questionable seed viability among seed pods. As seen in Figure 4, seeds collected in 2013 and 2014 did reach stage 5 faster than seeds collected in 2011 and 2012, with 31.00% of seeds reaching stage 5 in the 2013 seeds and 92.57%

for 2014 seeds while 27.04% of seeds reached stage 5 in the 2011 seeds and no seeds reached stage 5 in 2012 seeds.

Testing a Class II Sterile Hood

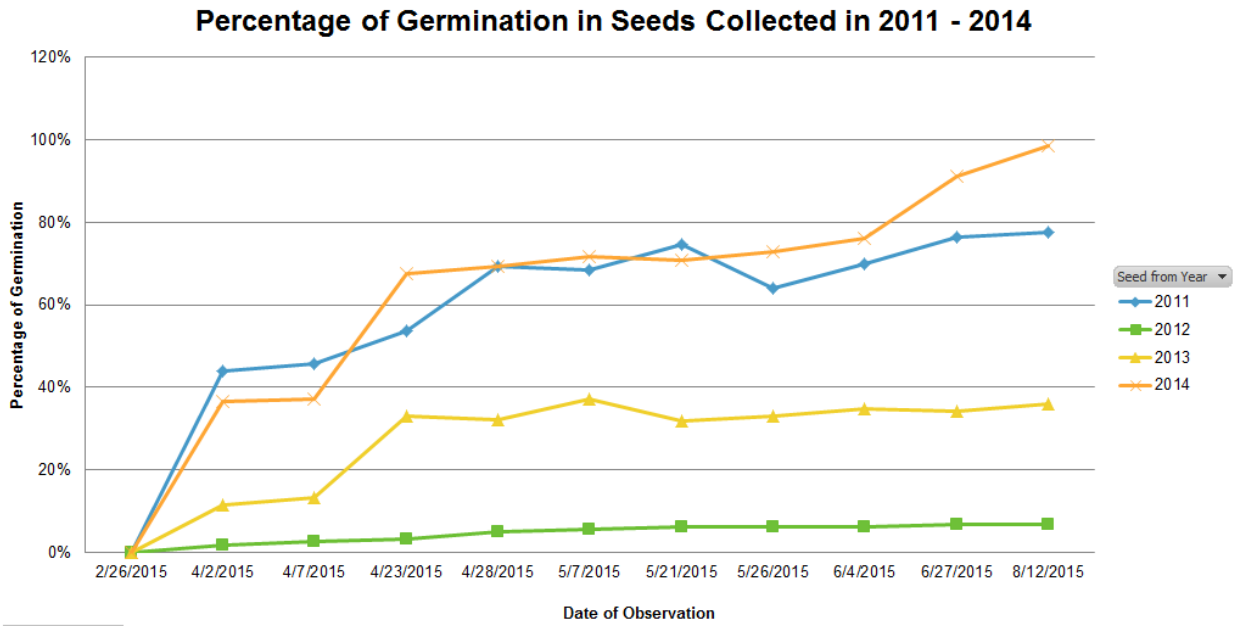
The results of the sterile hood showed that the hood was maintaining sterility at all speeds, with no contamination of any control or experimental vessels.

Comparing 3 Methods of Vernalization

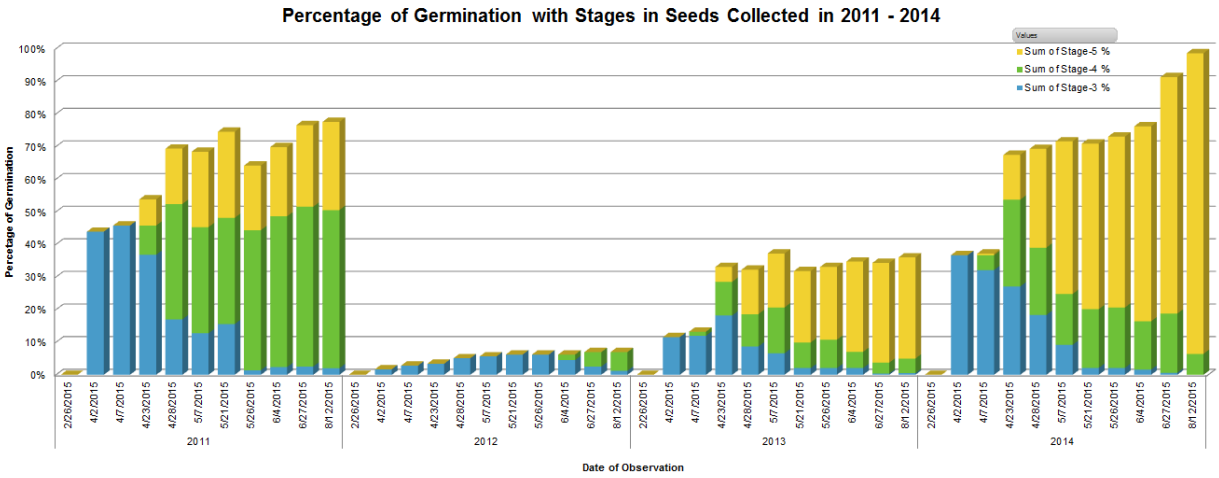
Of the three methods of vernalization, the soil storage method was the least effective with over 77.8% loss. Storage as bare roots was the second most effective but remained with a high level of loss at 62.5%. The storage of seedlings in their original culture vessels was by far the most effective with 0% loss (100% survival).

Fen Construction

We have created 4 fens in the past 3 years. The oldest fen had about 18 healthy 4-year-old plants, with the

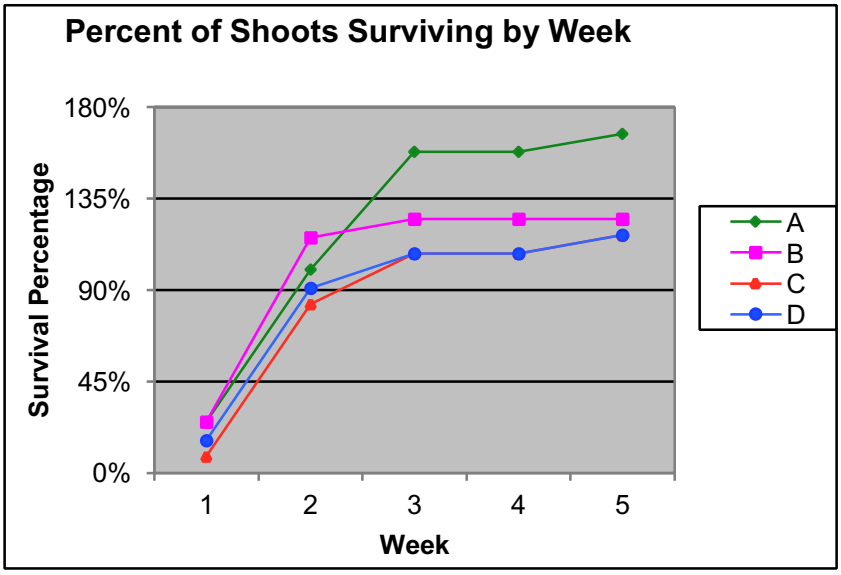


(Fig. 3)



(Fig. 4)

largest about 25 centimeters tall. This is near the size of a mature flowering plant and should be able to flower next year. This brings the time to maturity to within our expected 4-5 year range.



(Fig. 5) This graph shows the weekly percent survival of the 12 seedlings planted in each tray (A, B, C, and D). The percentages over 100 indicate when more than one shoot emerged from a single seedling.

Testing a novel hydroponic system

During the first 5 weeks of the hydroponics experiment, the humidity in the room averaged ~55%, the temperature averaged ~75ºF, and the pH in the reservoirs stayed at approximately 7. In the trays, the pH of the rock wool averaged 7.5 for the first 3 weeks but rose to 8 in the last 2 weeks. By the end of 5 weeks, the tallest seedlings in trays A, B, C, and D were 5.6cm, 4.5cm, 6.0cm, and 6.0cm, respectively. This is a substantially improved level of survival and growth rate over the standard approach of planting in soil, where there is a greater than 50% loss after transplantation and sprouting of leaves takes between 4-8 weeks. As shown in Figure 5, there appears to be to no correlation between the propagation medium used and the number of shoots that emerged. Half of the seedlings were transplanted to an artificial fen in August 2015, and the other half of the seedlings were placed in refrigeration for vernalization until next spring.

References Cited

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