INCREASING POPULATIONS OF KENTUCKY LADY'S SLIPPER ORCHID ON THE KISATCHIE NATIONAL FOREST: SEEDLING PRODUCTION AND OUTPLANTING TRIALS

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Abstract—The Kentucky lady's slipper orchid (*Cypripedium kentuckiense* C.F. Reed) is a tall, stately perennial plant with the largest flowers of any *Cypripedium* known. Its range includes much of the Southeastern United States, though it is rare throughout its range due to specific edaphic and climatic habitat requirements. In Louisiana, a few plants are located on four sites within the 600,000-acre Kisatchie National Forest (KNF). This effort is to increase populations of one of the most spectacular orchids native to the region. A high school student located a flowering orchid in the KNF, caused it to be pollinated, and later collected a fertile seed pod. A collaborative effort began between KNF, Southern Research Station, and Central Louisiana Orchid Society (CLOS) to restore the orchid on appropriate sites. Grants in 2006 from the Southwest Regional Orchid Growers Association and in 2007 from the U.S. Forest Service allowed CLOS to purchase plantlets grown from the collected seed pod. A research study is now underway to develop propagation protocols and compare effects of seedling age, fungal inoculation, and depth and season of planting on establishment success.

INTRODUCTION

A project to restore one of the rarest and most spectacular orchids native to the west Gulf Coast region began with the curiosity of a high school student. Inspired by the beauty of lady's slippers in a book on orchids, Kevin Allen, then a high school student, was determined to locate the Kentucky lady's slipper (*Cypripedium kentuckiense* C.F. Reed) orchid that was known to exist in the Kisatchie National Forest (KNF). With help from KNF botanists, he located two small populations and self-pollinated plants when in flower (fig. 1). After 3 years, he obtained a seed capsule. This seed pod was sent to Spangle Creek Labs in Bovey, MN, who are specialists in *Cypripedium* seed germination. Seeds from the collection were viable and Spangle Creek Labs produced small plantlets which could be used in the effort. Kevin Allen approached the KNF to determine their interest in a restoration project. Lacking the technical expertise to grow the plantlets to plantable size, Southern Research Station (SRS) and Central Louisiana Orchid Society (CLOS) personnel were approached for assistance in the effort. Thus began a collaborative effort to increase Kentucky lady's slipper orchids on KNF (Barnett 2008).

Literature specific to *C. kentuckiense* largely accesses terminology of the species because there has been debate and confusion among plant taxonomists about its proper classification (Atwood 1984). For years it had been classified as *C. calceolus* var. *pubescens* or a natural hybrid. An isozyme study has confirmed that *C. kentuckiense* is a distinct species (Case and others 1998). Based on herbaria collections, it ranges from eastern Texas and Oklahoma to Kentucky and Virginia (Atwood 1985). However, most States where the species now occurs have designated *C. kentuckiense* as endangered, threatened, special concern, or otherwise imperiled and vulnerable to extinction (Allen and others 2004, Case and others 1998). The population continues to decline from loss of habitat and human predation (Cash 1991).

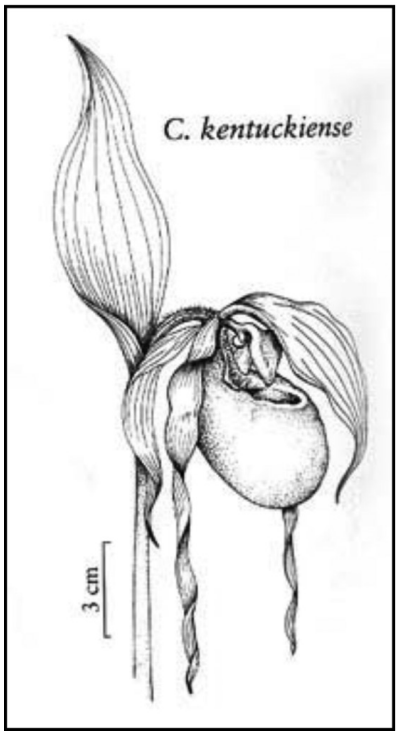

Figure 1—*Cypripedium kentuckiense* from "The Flora of North America" Volume 26. Copyright © 2000 Flora of North America Association.

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Information on the propagation and reestablishment of this terrestrial species is sparse. Orchid enthusiasts generally grow orchids of the epiphytic type in greenhouses and grow relatively few terrestrial species even in garden situations. Some research trials have been conducted with other species of the *Cypripedium* genus. Huber (2002) has successfully established *C. montanum* by mixing seeds with a carrier such as forest soil, sugar, or cracked corn prior to sowing in forest openings. The most significant reestablishment effort of a *Cypripedium* species has been the effort by Ramsey and Stewart (1998) in the United Kingdom with *C. calceolus*. They have had some limited success with direct seeding, but have studies underway to pot seedlings for planting in the wild when grown to an appropriate size.

SEED CAPSULE COLLECTION

One of the orchids self-pollinated by Kevin Allen produced a seed capsule. Seeds from either green or dry capsules can be used to produce seedlings via in vitro methods (Cullina 2004); Allen collected the capsule in its dry condition. He sent the dried fruit to Spangle Creek Labs (www.spanglecreeklabs.com) within a few weeks after collection.

SEED GERMINATION AND PLANTLET DEVELOPLMENT

Orchid seeds have essentially no stored food resources and require external sources of nutrition for germination. In nature, they obtain nutrients and energy by absorption from a symbiotic fungus which invades the seeds (Ramsey and Stewart 1998). The term symbiotic is usually used for the relationship between a fungus and orchid; however, no benefit to the fungus has been found. Germination proceeds in *in vitro* culture where nutrients are supplied in a liquid solution or gel medium. Different species of orchids, even within the same genus, require media of different composition (Steele 2007).

Both media and seeds require sterilization; otherwise, the cultures would quickly be overrun by competing organisms such as fungi and bacteria. Steele (1995, 2007) outlines procedures for sterilizing orchid seed; methods used by Spangle Creek Labs are not proprietary and can be reviewed by contacting the lab (www.spanglecreeklabs.com). The sterilized seeds are then moved to a specialized agar medium in a flask for germination and development under aseptic conditions. These flasks must remain under dark conditions for the entire germination period.

After 3 to 4 months on specialized media in flasks, the embryos begin to swell and rupture the seed coat, and a white body known as the protocorm is formed. Protocorms are transferred from the sowing flasks to fresh media when sufficient size to be handled by a forceps without damage. To minimize browning of the plants that may result from the handling, protocorms are placed at a density that allows good root development to take place with no requirement for additional transfers that might damage them (Ramsey and Stewart 1998). Flasks are kept in the dark throughout this period to encourage root rather than shoot growth. It will take up to 6 months for the plantlets to develop to a size for deflasking.

Many species of *Cypripediums* require chilling to hasten germination and shoot development (Curtis 1943). Since *C. kentuckiense* has a more southern range than many of these orchids; extent of dormancy in this species is not understood. Upon removal from the flasks, the plantlets are thoroughly rinsed in water and placed in sealed plastic storage conditions with enough water to barely cover the bottom of the container. The containers are then placed in a refrigerator for about 4 months to ensure the seedlings are properly vernalized (Steele 2007).

TRANSFER OF PLANTLETS TO TRAYS FOR GROWTH

Once problems of collecting and sowing seed and maintaining plant growth and development *in vitro* have been resolved, the grower now must transplant these seedlings successfully to a nursery environment and ultimately establish them in forest conditions. Many plants grown *in vitro* are difficult to transfer into compost-type medium as they are acclimatized to high levels of humidity.

In our study, plantlets received from Spangle Creek Labs had buds near 1 cm long and roots 4 cm or more in length (fig. 2). These were planted in trays filled with media consisting of compost, commercial potting soil (PRO-MIX®) and sand. The 200 plantlets received in late May 2006 were distributed to several growers and the potting medium, seedling culture, and environmental exposures varied by grower in an effort to determine guidelines for *C. kentuckiense* seedling production. Although initial shoot growth was excellent, survival of seedlings during the next several months was poor.

Most growers initially started plantlets in greenhouse environments, but it became apparent that summer greenhouse temperatures were excessive and mortality

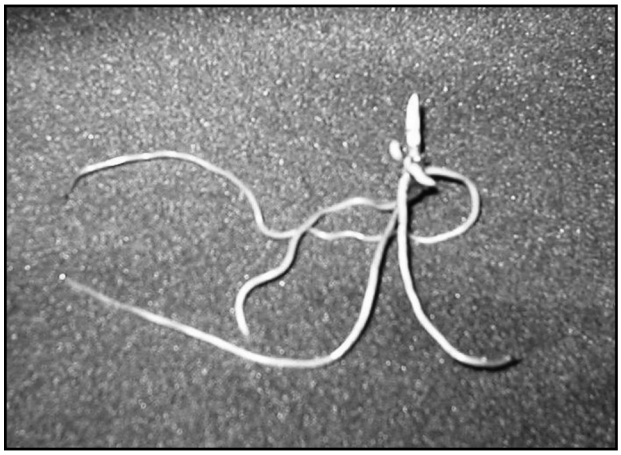

Figure 2—Plantlet of *Cypripedium kentuckiense* after 6 weeks in potting medium showing initial shoot development and root system.

or dormancy occurred. Growers then moved the plants to outside, shaded sites.

SEEDLING PRODUCTION

Based on observations through 2006, we concluded that (1) greenhouse temperatures during the summer are too high for these terrestrial orchids, (2) seedlings develop better in a dappled shade environment, and (3) a peat-sand mix worked well for plant development. Seedling shoots died early in the summer if the plants were under stress, either by high temperatures or droughty conditions. If the stress was not severe, many of the seedlings became dormant with the root system still alive. These dormant seedlings began growth again early in the next spring.

In 2007, 700 additional seedlings from the same seed source and lot were purchased. These, again, were distributed among growers. A commercial potting mix (PRO-MIX®) with sand (2 to 1 ratio) was used by all growers, and seedlings were grown in shaded outdoor environments. Percentage of seedlings alive at the end of the first-growing season improved over the previous year.

STUDY TREATMENTS IN FIELD PLANTINGS

On December 18, 2007, and March 6, 2008, seedlings grown from the 2006 and 2007 shipments of plantlets were outplanted in field trials in the Catahoula Ranger District of the KNF. Four replications of five seedlings per plot were planted along a stream near a small group of native Kentucky lady's slipper orchids. Plants that serve as indicator plants for orchid sites are American beech (*Fagus grandifolia*), eastern hophornbeam (*Ostrya virginiana*), horsesugar (*Symplocos tinctoria*), and witch hazel (*Hamamelis virginiana*) in the overstory and an abundance of poison ivy (*Rhus radicans*) and broad beechfern (*Thelypteris hexagonoptera*) in the understory (Allen and others 2004). Before planting, number of roots and shoots of each seedling was recorded (table 1).

For outplanting seedlings, we followed recommendations Cullina (2008) developed for transplanting divisions of *Cypripedium* plants. The procedure requires first working compost or duff into the planting site to a depth of 6 to 8 inches, then developing a cavity in the soil several inches deep, placing the root system so the seeding bud is near the groundline surface, and replacing soil around the seedling with the bud at the surface. The planted seedlings were covered with mulch to protect the plant and conserve moisture.

Results from the December planting

The December planting compared seedling age (seedlings from 2006 and 2007 crops). Seedlings from the 2006 crop were received from Spangle Creek Labs in late May and transplanted into trays of potting media in early June. Lack of knowledge of growing culture resulted in considerable mortality, but enough from the 200 purchased remained to plant after a second-growing season in the trays of potting mix. Seedlings from a 2007 crop were obtained in April and were transplanted into trays with a PRO-MIX®-sand mixture.

It is interesting to note that seedlings from the 2007 crop had higher numbers of roots and shoots than those from the 2006 crop (fig. 3). This suggests that holding the 2006 seedlings through 2007 in the same trays without additional nutrients reduced seedling vitality. Steele (2007) reports lack of success in growing *C. arietinum* seedlings in any sort of peat-based mix for longer than one season.

Seedling survival was measured April 1 and July 7, 2008 (table 1). Survival in April was 70 and 50 percent for seedlings

Table 1—Seedling characteristics and survival of various treatments when planted on December 18, 2007, and March 6, 2008

	Characteristics at planting		Survival	
Planting variable	Roots	Shoots	4/1/08	7/7/08
	---------- *number* ----------		---------- *percent* ----------	
	December 18, 2007, planting			
2006 crop	6.5[a]	1.0	70	25
2007 crop	8.3	1.3	50	15
	March 6, 2008, planting			
2007 unfertilized	8.4	1.3	85	15
2007 fertilized[b]	8.1	1.2	65	10

[a] The numbers represent an average of four replications of five seedlings each.

[b] Slow-release nutrients (1 teaspoon of Osmocote 19-6-12) were applied at each planting spot after each seedling was planted.

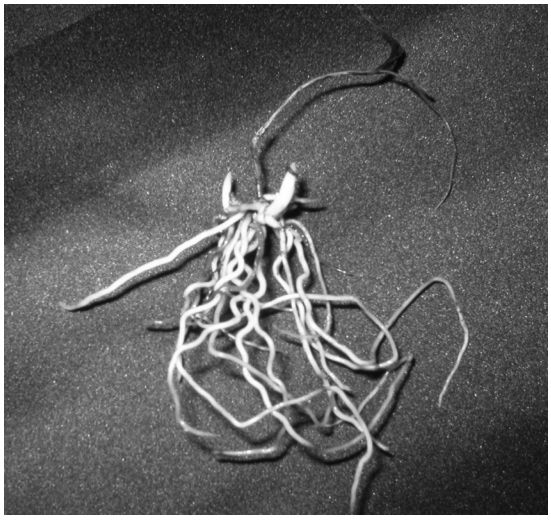

Figure 3—*Cypripedium kentuckience* seedling lifted after 8 months in potting medium and before being outplanted into the Kisatchie National Forest. Note the dead shoot retained after the seedling became dormant and the two developing shoots.

from the 2006 and 2007 crops, respectively. However, 3 months later, survival measured was only 25 and 15 percent for the same treatments. Rapid increases in seedling mortality are likely related to a combination of droughty conditions at the planting site and the shallow planting of seedling root systems. At planting, the seedlings were small with poorly developed root systems.

Results from the March planting

Variables evaluated in March were fertilization applied as the seedlings were planted—fertilized and nonfertilized. Fertilization was accomplished by scattering 1 teaspoon of Osmocote® 19-6-12 to the planting site after the seedling was planted. No data were available to guide the rate of application; but, it was believed that some level of fertilization would help seedling growth and potential flowering. Results indicated that the treatment rate, as applied, was too high and adversely affected survival (table 1).

CONCLUSIONS

This restoration effort resulted from a high school student's curiosity about one of the South's rarest and most spectacular orchids—*C. kentuckiense*. After several years' efforts in locating and pollinating flowering plants and with assistance of a *Cypripedium* specialist, plantlets became available for potential seedling development and reintroduction studies. Thus began a collaborative effort among Kevin Allen, Spangle Creek Labs, U.S. Forest Service's KNF and SRS, and the CLOS.

Quickly, it became apparent that little information exists to propagate, culture, and outplant this terrestrial orchid species. In fact, information about the entire *Cypripedium* genus is sparse. Largely by trial and error, information is being developed to provide guidelines to those interested in orchid restoration. Current trials indicate that plantlet development into appropriate seedling size and condition requires peat-sand medium that receives a low level of nutrients. Data also indicate that seedlings that perform well in the field may take 1 or more years to develop under nursery conditions.

Gains are being made in developing cultural practices appropriate for the species; however, additional studies are needed to provide meaningful guidelines for restoration. Studies are now underway to evaluate fertilization needs, seedling size, fungal inoculation, and planting depth. Another aspect of restoration is to quantify sites appropriate for reintroduction of the species. Indicator species provide some direction in site selection, but additional efforts are needed to determine soil types and pH and light conditions most suited for Kentucky lady's slipper establishment.

In an effort to provide future planting stock, Kevin Allen, now a science teacher at Captain Shreve High School is beginning an effort to refine seed germination and plantlet development technology so that seedlings can be produced in sufficient quantities and at reasonable costs so that restoration efforts may continue. This effort will be accomplished as honors chemistry projects and will be supported by grant funding from the U.S. Forest Service and orchid organizations.

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