Proceedings of The National Conference On Undergraduate Research (NCUR) 2016 University of North Carolina Asheville Asheville, North Carolina April 7-9, 2016

Habitats of Puttyroot (Aplectrum hyemale) and Cranefly (Tipularia discolor) Orchids: A Comparison of Two Wintergreen Species in Western North Carolina

Tyler Clabby Environmental Studies The University of North Carolina Asheville One University Heights Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Irene Rossell

Abstract

Puttyroot orchid (*Aplectrum hyemale*) and cranefly orchid (*Tipularia discolor*) are two wintergreen species found across much of the eastern United States. Like other wintergreens, both species have a unique phenology. In the winter, when the canopy is clear of deciduous leaves, they unfurl a single leaf and photosynthesize with little competition. By spring, the leaves die back and a single flowering stalk emerges in summer. There have been marked declines of both orchids across their northern range, yet both seem to be thriving in the southern Appalachians. Due to conservation pressures in northern states, most published studies on both species have focused on this region; subsequently, little is known about their habitat in the southern Appalachians. The purpose of this study is to investigate the abundance and habitat requirements of these two species in order to contribute to their conservation in the southern mountains. The Coleman Boundary of Pisgah National Forest was chosen as a study site due to prior knowledge of the species being found there. Data on population size, light levels, leaf chlorophyll, number of flowering stems, surrounding vegetation, slope aspect, and soil characteristics were gathered the determine the extent that the two habitats overlapped. This study showed that the two species have overlapping habitats with similar metrics for the variables collected. The mean percent full sun for puttyroot orchid was 46.4 and for cranefly orchid it was 46.7, showing that both species prefer 50% full sun habitats.

Keywords: Puttyroot Orchid, Cranefly Orchid, Habitat

1. Introduction

Puttyroot orchid (*Aplectrum hyemale*) and cranefly orchid (*Tipularia discolor*) are indigenous to much of the eastern half of continental North America. They are listed as rare, threatened, or state-endangered within much of their northern range. These species have a unique life history among forest understory plants. During the fall (October) they unfurl a single leaf that photosynthesizes throughout the winter. In the spring, the leaves die back and a single flowering stalk emerges⁷. By waiting until late fall to produce a leaf, wintergreen orchids gain the advantage of photosynthesizing with minimal competition from the tree canopy. Little is known about the habitat of these plants in the southern Appalachians, as the majority of research has been done in their northern range. Since the leaves do not emerge until fall, both species are easily overlooked during routine floral surveys that take place in spring and summer.

Puttyroot and cranefly orchids are found primarily in mesic deciduous forests. Populations are generally scattered and small. Soils are generally moist, but not too wet, and swampy areas are less than ideal⁶. Neutral soils are preferred, though they may tolerate slight acidity. Light, rather than soil nutrients, may be the main limiting resource for most

forest understory herbs in late successional forest stands². However, since these orchids grow in winter when the canopy is mostly open, competition for light is less intense, so soil characteristics (e.g., pH, Ca, organic matter) may play a larger role in their distribution¹.

The objectives of this study are to quantify specific habitat requirements of puttyroot and cranefly orchids in the southern Appalachians and determine the extent that their niches overlap. The results will contribute to their conservation across their southern range.

2. Methods

This study took place in the Coleman Boundary of Pisgah National Forest, near Barnardsville, NC. The upper ridges of the Coleman Boundary extend to Craggy Gardens in the Black Mountain Range along the Blue Ridge Parkway. Elevations in the study area ranged from 2750 to 3000 feet above sea level. The Coleman Boundary was chosen to for this study because prior observations indicated both species of orchids occurred in this area. Tulip poplar (*Liriodendron tulipifera*) is the dominant overstory tree throughout the forest, and there is evidence of past logging.

Overall orchid abundance across the study site was determined in early March 2016 with a series of 50, $100m^2$ circular plots. Each plot was centered on a randomly selected point and surveyed for puttyroot and cranefly orchids. More intensive data on habitat and plant characteristics were collected in 26 patches of each species between mid-December 2015 and late February 2016. For puttyroot orchids, 26 random points were selected across the study area and the closest patch of puttyroot to each point was quantified (a patch was defined as ≥ 1 plant growing in close proximity). Since cranefly orchids were scattered much more widely throughout the forest and were more difficult to find, 26 patches of cranefly orchids were quantified as they were encountered in the study area.

In each of the 52 patches the number of orchids in the patch was counted, the azimuth was determined, the nearest overstory tree identified and its DBH measured, and the distance from the tree to the closest orchid was measured. Photosynthetically active radiation was measured above each orchid leaf using a LiCor Li-250A light meter. A light measurement was also taken in a nearby open area light could be quantified as a percent of full sunlight. A handheld Opti-Sciences CCM-200 Plus chlorophyll meter was used to take one reading of chlorophyll (CCI) near the center of each leaf. Reproductive structures and evidence of herbivory were noted for both species. Soil samples were taken in 18 of the random patches (10 for puttyroot and 8 for cranefly). Soil samples consisted of ten cores (2.5cm x 10 cm deep) that were homogenized, air dried, and sent to Waters Agricultural Laboratory (Warsaw, NC) for analysis of pH, Ca, Mg, P, K, CEC, and humic matter.

Data on percent full sun and leaf chlorophyll were averaged for all leaves in each patch. Student t-tests (alpha = 0.05) were performed on the 26 patch averages for each species to determine whether sunlight or leaf chlorophyll differed between the two species. T-tests were also used to compare the soil characteristics of each species.

3. Results

Referring to Figures 1 and 2, across the study site, the two orchids had the same overall abundance (~ 150 plants, density of 0.03 plants/ m^2). However, the species had different distribution patterns. Cranefly orchid had a patchier distribution, occurring in only 50% of the 100 m^2 plots. When small patches of cranefly orchid were quantified, 50% of patches had only 1 plant. In contrast, puttyroot occurred in 75% of the 100 m^2 plots, and when small patches were quantified, 57% contained up to 5 plants. This led to a slightly larger patch size for puttyroot (5.5 plants, compared to 4 for cranefly orchid).



Figure 1. Overall abundance of puttyroot orchid and cranefly orchid in 50, 100-m² plots across the study site.



Figure 2: Abundance of puttyroot orchid and cranefly orchid in 26 random patches of each species

Table 1 shows the habitat data for small patches of puttyroot and cranefly orchid. The two species occurred in remarkably similar habitats. Both species occurred in generally south-facing locations. The mean distance from an orchid to the closest overstory tree (2.4 m for puttyroot and 2.2 m for cranefly) was virtually the same, as was the DBH of the overstory tree. Another interesting similarity between the two was the nearly identical light conditions (46.4% full sun for puttyroot vs. 46.7% for cranefly). However, mean leaf chlorophyll differed significantly between the species ($P \le 0.001$), with leaf chlorophyll levels in puttyroot orchid twice as high as those in cranefly orchid.

The species composition of the closest overstory trees was similar for both species: Tulip poplar (*Liriodendron tulipifera*) comprised 65% of nearest trees to puttyroot patches and 57% of nearest trees to cranefly patches. Other tree species (American beech, *Fagus grandifolia*; sweet birch, *Betula lenta*; Cucumber tree, *Magnolia acuminata*; and Chestnut oak, *Quercus prinus*) occurred in low frequency.

Table 1. Habitat data for small j	patches of puttyroot and	cranefly orchids
-----------------------------------	--------------------------	------------------

	Puttyroot	Cranefly
Number of random patches	26	26
Total number of plants in all patches	144	103
Mean number of plants/ patch	5.5	4.0
Mean azimuth (degrees)	168	181
Mean DBH of closest overstory tree (cm)	44.2	40.9
Mean distance to closest overstory tree (m)	2.4	2.2
Mean leaf chlorophyll (CCI)	40.5	23.1
Mean percent full sun	46.4	46.7

Table 2 references the soil characteristics studied as well as their averaged results across the study area. None of the soil characteristics differed significantly between patches of puttyroot and cranefly orchids. However, soil pH showed a strong trend toward greater acidity in patches of cranefly orchid (P = 0.058). Only ten puttyroot patches and eight cranefly orchid patches were analyzed for soil characteristics. A larger sample size might have yielded more differences.

Table 2. Mean soil characteristics for patches of puttyroot and cranefly orchids

Variable	Puttyroot	Cranefly	P-values
pH	6.2	5.8	0.058
Ca (lbs./acre)	3154	3565	0.714
Mg (lbs./acre)	542	479	0.556
P (lbs./acre)	16	14	0.216
K (lbs./acre)	459	386	0.112
CEC (meg/ 100g)	12	10	0.412
Humic Matter (%)	0.54	0.63	0.177

4. Discussion

The two species of wintergreen orchids occurred in very similar habitat conditions and in equal numbers in this study, but were distributed differently in the forest. Cranefly orchid occurred less frequently but was more likely to occur as single plants. However, one plot was found with 39 plants, compared to a maximum of 17 plants for puttyroot orchid.

One reason for the large patch might be a greater propensity for cranefly orchid roots to produce clonal offspring⁵. Another reason might be related to substrate, as a strong connection between woody debris and cranefly seed germination has been reported. Each group of cranefly orchids could be clustered based on current or previous woody debris in the area⁵. Throughout the study site there were more observations of cranefly orchid growing on old logs or stumps, compared to puttyroot orchid. Although not statistically significant, high levels of humic matter in soils with cranefly were observed. This could be related to the degraded woody debris.

Both species showed a similar preference for \sim 50% full sunlight and grew a similar distance to similarly sized trees. Since the orchids were shown to lack plasticity when dealing with light conditions, it can be presumed that filtered light might allow sufficient sunlight for photosynthesis while protecting the leaves from intense winter sunlight.

Leaf chlorophyll was the only variable that differed significantly between the two species. Puttyroot leaves contained twice as much chlorophyll as cranefly, which was interesting considering that they occurred in the same light environment and in similar soils. It was observed that overall, larger leaves tended to have more chlorophyll than smaller leaves, and puttyroot leaves tended to be larger than cranefly leaves. Winter orchids appear to follow a life history similar to spring ephemerals, with little photosynthetic plasticity⁹. The noninvasive optical method used

in this study has been shown to produce results comparable to extracting leaf chlorophyll⁶. Differences in chlorophyll content could be related to another environmental variable such as soil nitrogen, which was not measured in this study.

No residual summer flowering stalks were observed for cranefly orchid whereas five puttyroot flowering stalks were observed over the course of this study. Sexual reproduction appears to be a less important means of colonization than asexual reproduction for both species. One of the reasons that cranefly orchids may not have flowered the previous summer is because there is a clear correlation between size of plant and flowering, with larger plants being more likely to flower than smaller ones⁸. Most of the cranefly orchid leaves observed in this study were smaller than puttyroot orchid leaves. In the future, it would be interesting to quantify leaf area to see if it correlates with the production of flowering stalks.

Herbivory by deer was observed on several cranefly orchids, but not on puttyroot orchids. Others have reported that cranefly orchids are predated upon by deer or rodents⁸. This raises the question of whether deer have contributed to the patchy distribution of cranefly across the Coleman Boundary, and what impacts a larger deer population might have on this plant species.

5. Conclusion

Puttyroot and cranefly orchids were shown to occupy nearly identical habitats in this study site. Cranefly orchid exhibited a patchier distribution, lower leaf chlorophyll, no evidence of flowering stalks, and occasional herbivory by deer, compared to puttyroot. To maintain both species in the landscape, land managers should manage the forest for optimal light conditions, monitor impacts of deer herbivory, and leave woody debris for colonization by cranefly orchids.

This research further solidifies evidence of wintergreen species lacking photosynthetic plasticity. This would mean that wintergreen species have similar photosynthetic traits to spring ephemerals.

6. Acknowledgments

I extend thanks to the following for field assistance: Peter Menzies, Lilian Lovingood, Grace Schermerhorn, Grace Rossell, and Reed Rossell. I would also like to thank Irene Rossell, who I'm eternally grateful to for helping me throughout the entirety of this project, from fieldwork to paper editing.

7. References

1. K..J. Elliot and others, "Long-term patterns in vegetation-site relationships in a southern Appalachian forest," Journal of the Torrey Botanical Society 126 (1999): 320.

2. F.S. Gilliam and M.R. Roberts, The Herbaceous Layer in Forests of Eastern North America (New York: Oxford UP, 2003).

3. Massachusetts Division of Fisheries and Wildlife, "Natural Heritage and Endangered Species Program n.d. Cranefly orchid *Tipularia discolor*, <u>http://www.mass.gov/eea/docs/dfg/nhesp/species-and-</u>conservation/nhfacts/tipularia-discolor.pdf.

4. Pennsylvania Natural Heritage Program, "n.d. Cranefly orchid,"

http://www.naturalheritage.state.pa.us/ccvi/cranefly%20orchid.pdf.

5. H.N. Rasmussen and D.F. Whigham, "Importance of woody debris in seed germination of *Tipularia discolor* (Orchidaceae)," American Journal of Botany 85 (1998): 829.

6. A.D. Richardson and others, "An evaluation of noninvasive methods to estimate foliar chlorophyll content," New Phytologist 153 (2002): 185.

7. J.A. Richburg, "*Aplectrum hyemale* (Muhl. Ex. Willd) Nutt, Puttyroot: Conservation and Research Plan for New England," New England Plant Conservation Program. New England Wildflower Society, 2003, http://www.newenglandwild.org/docs/pdf/aplectrumhyemale.pdf.

8. A.A. Snow and D.F. Whigman, "Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae)," Ecology 70 (1989): 1286.

9. D.T. Tissue and others, "Photosynthesis and carbon allocation in *Tipularia discolor* (Orchidaceae), a wintergreen understory herb," American Journal of Botany 82 (1995): 1249.