Nutrient availability of white lady's slipper orchids(*Cypripedium candidum*) affects presence of mycorrhizal partners

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Introduction

The Orchidaceae family is the world's largest flowering plant family with approximately 27,135 species (Jones 2005). Orchids are very niche oriented organisms and belong to three main growth habitats: terrestrial species, lithophytes, and epiphytes (Dearlaney 2007). Within these habitats, orchids share important relationships with their fungal partners. Orchids produce dust-like seeds, with little nutrient availability, and so rely on fungi to supply the seed with carbon and and other limiting nutrients for germination and seedling development (Bernard 1904). As adults some photosynthectic species are known to continue to rely upon fungal relationships, but the extent of their reliance on fungi is not always clear (Gebauer and Meyer, 2003). We studied *Cypripedium candidum*, the white lady's slipper orchid, a terrestrial species belonging to the genus *Cypripedium*. *Cypripedium* is a genera of approximately 45 species found in the temperate Northern Hemisphere (Shefferson et al. 2007). *C. candidum* is usually found in wet prairies, bogs, and fens. *Cypripediums* are often locally rare, but have a wide geographic distribution. Dormancy is common in *Cypripedium*. During dormancy plants are able to survive without producing above ground growth for years (Kull 2002). This characteristic may suggest continued use of mycorrhizae as sources of carbon, nitrogen, phosphorus and other nutrients by mature plants (Shefferson et al. 2007).

Figure 1. C. Candidum plant native to the Chicago Region

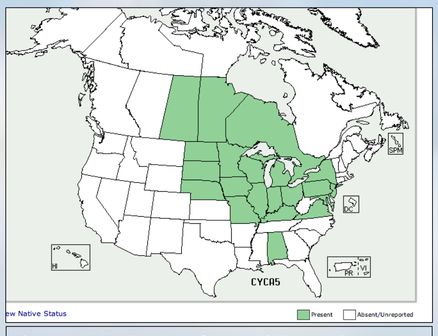


Figure 2. Distribution of *C. candidum* in the U.S. and Canada (http://plants.usda.gov/maps/large/CY/CYCA5.png)

Research Question

Does the presence of mycorrhizal fungi in the white lady's slipper orchid, *C. candidum,* affect soil nutrient availability across habitats in the Chicago Region?

Hypothesis

If there is evidence of mycorrhizal colonization in root tissue, then sites examined with the highest rates of colonization will also have the highest nutrient availability.

Methodology

Data Collection

Four sites across the Chicago Region were chosen to provide variety in habitat.

- We used GPS coordinates, collected by the Plants of Concern program, of known plant populations.
- Using Arc-GIS buffer, we created buffer zones one meter and three to five meters away from GPS coordinates and random sampling points were generated.
- 20 near soil samples were taken within the one-meter zone and 20 far samples were taken from the three to five meter zone.
- We plated root tissue found in soil samples for observation of mycorrhizal colonization.

Sample Preparation

- We air-dried all collected soil samples.
- We cut root samples into 2.54 cm segments, stained, placed on slides and cased with a poly-vinyl alcohol.

Nutrient Analysis

- We performed KCL extractions using approximately 10 mL of KCL and 1 gram of each of the 150 soil samples.
- We prepared reagents for nitrate, phosphorus, and ammonium reactions.
- We made Concentration standard plates for each nutrient by aspirating the reagent in a 96 well plates with a micropipette, waiting for a reaction to occur and reading them at nutrient specific wavelengths on an Epoch spectrophotometer.
  - A standard curve was made on excel using concentration of the standard on the y-axis and absorbance reading on the x-axis.
  - We then calculated the concentration using the equation: Concentration (mg/g) =ppm x (total volume of extract / weight) x (total volume in well/ aliquot volume
  - Using the KCl extractions, we performed nutrient analyses for nitrate, ammonium, and phosphorus using reagents and same methodology used to prepare the standard plates.

Statistical Analysis

- We performed One-Way Analysis of Variance tests across all sites for each nutrient to test the significance of site on nutrient availability. Confidence Intervals at 95% were also performed. Both test were performed using the statistical program *R.*

Fungal Observations

- We observed prepared root segments using a compound microscope for fungal colonization inside cortical cells.
- We recorded pelotons that were less than 75% digested.

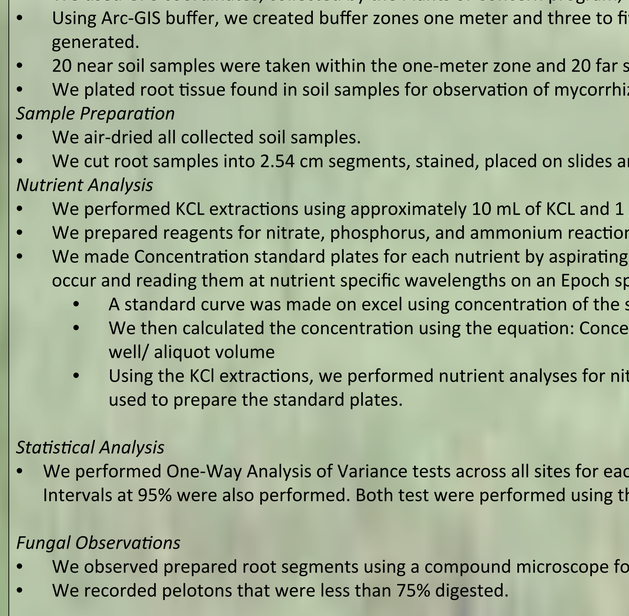


Figure 2.5. Student, Geralle Powell, measuring samples to be placed in the LECO dry combustion system.

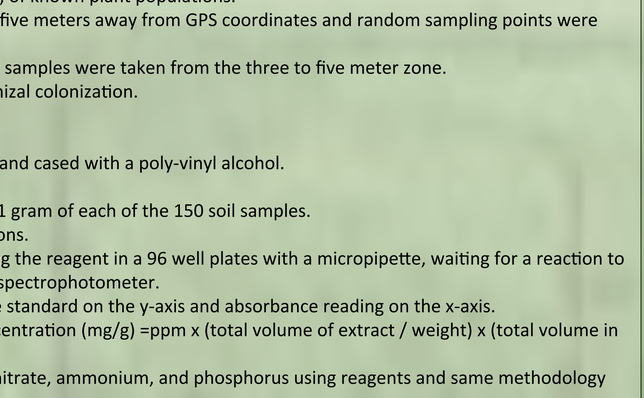


Figure 3. Buffer Zones are represented. Green represents 1 m zone, white represents 3 m zone and pink represents 5 m zone

Results

Nutrient Analyses

- We took averages of total nutrient concentration for carbon, nitrogen, phosphorus, ammonium, nitrate, total carbon and nitrogen, and available organic nitrogen.
- Our results showed that GB had the highest concentrations of all nutrients, except available organic nitrogen. RF surpassed GB only in total available nitrogen.
- Chicago Ridge had the lowest level of nutrients observed, with the exception of average ammonium concentration.
- We performed One-Way Analysis of Variance tests across all sites for each nutrient to test the significance of site on nutrient availability. Confidence Intervals at 95% were also performed. Both test were performed using the statistical program *R*.
- For all nutrients, excluding phosphorus differences in concentrations across sites were significant (Figure 5).

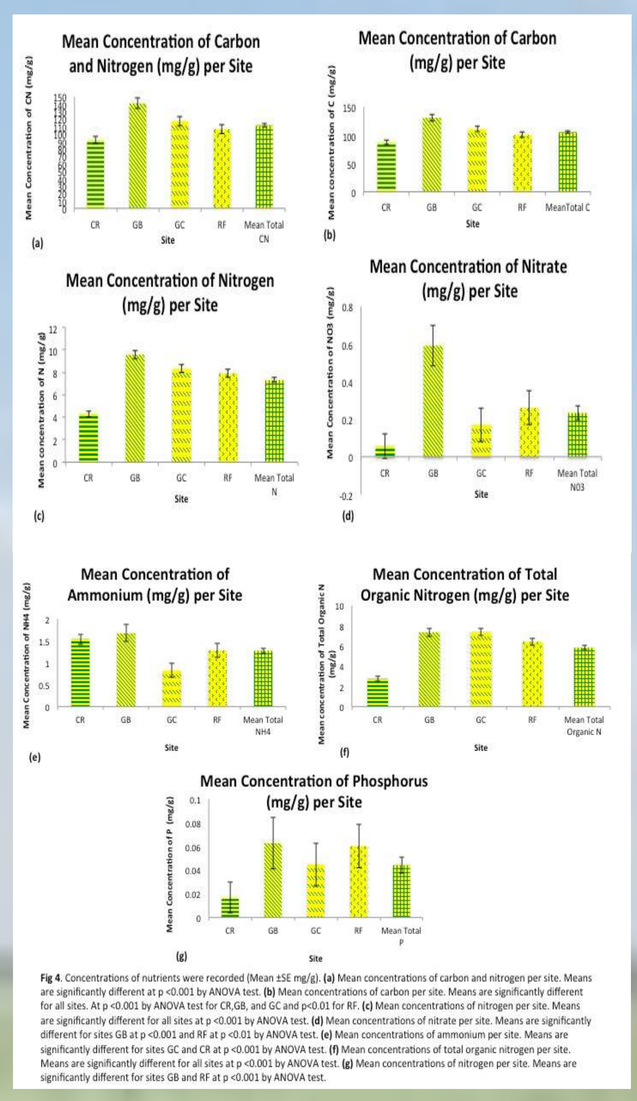


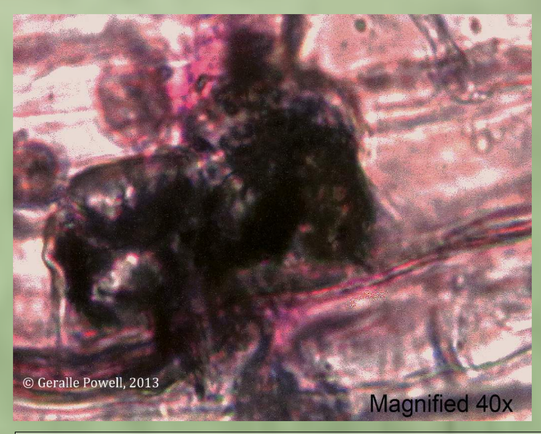
Fig 4. Concentrations of nutrients were recorded (Mean ±SE mg/g). **(a)** Mean concentrations of carbon and nitrogen per site. Means are significantly different at p <0.001 by ANOVA test. **(b)** Mean concentrations of carbon per site. Means are significantly different for all sites. At p <0.001 by ANOVA test for CR,GB, and GC and p<0.01 for RF. **(c)** Mean concentrations of nitrogen per site. Means are significantly different for all sites at p <0.001 by ANOVA test. **(d)** Mean concentrations of nitrate per site. Means are significantly different for sites GB at p <0.001 and RF at p <0.01 by ANOVA test. **(e)** Mean concentrations of ammonium per site. Means are significantly different for sites GC and CR at p <0.001 by ANOVA test. **(f)** Mean concentrations of total organic nitrogen per site. Means are significantly different for all sites at p <0.001 by ANOVA test. **(g)** Mean concentrations of nitrogen per site. Means are significantly different for sites GB and RF at p <0.001 by ANOVA test.

Nutrient	Mean	SE	CI 95%	Pr(>F)
Phosphorus	0.04	0.01± 0.01	p>0.05	
Carbon	105.30	2.09± 4.13	P< 0.001	
Total Org N	5.82	0.21± 0.41	P< 0.001	
Nitrogen	7.30	0.21± 0.41	P< 0.001	
TotalCN	111.10	2.60± 4.48	P< 0.001	
Nitrate	0.23	0.04±0.070	P< 0.001	
Ammonium	1.27	0.07± 0.12	P< 0.001	

Figure 5. Means of Concentration, standard error of means, confidence intervals at 95%, and p-value are represented.

Fungal Observations

- Fungal Colonization at all sites was extremely low with a colonization rate of 0.4%.
- GB had the highest rate of colonization with a total rate of 7.5%. Russell Fen had 0.1% colonization and GC had 0.4 % colonization.
- We did not observe any fungal colonization for CR.



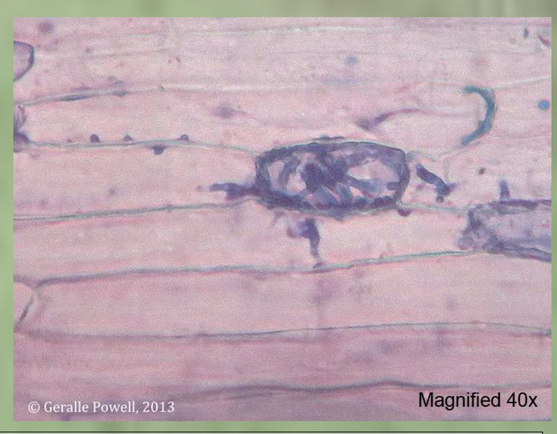


Figure 6. Mycorrhizal colonization in root cortical cells in represented. Left represents a partially digested peloton at site GB. Right represents a peloton more than 75% intake at site RF.

Discussion

Mycorrhizal fungi are necessary for germination for all orchid species. As orchids age they change their fungal partners. While some myco-heterotrophic orcids remain totally dependent on their fungal partners, photosynthetic orchids retain a limited dependence on their fungal partners. Research is needed to examine on the extent of the fungal relationship during adulthood. *C. candidum* (Sheffereson 2007). Our results show that GB had both the highest rate of colonization and nutrient concentration, while CR had both the lowest rate of colonization and lowest nutrient concentrations. This suggests that there is a positive relationship between fungal colonization and nutrient assimilation. Because total colonization rate across all four habitats was low, more data should be collected. Since samples were taken during the spring season after plants had reproduced and acquired new growth, it is possible that plants may have used all their accessible nutrients for these processes, including digestion of the fungal hyphea that colonized their roots in the previous growing season. More sample collection and data analysis should be taken during the growing season in the late fall and early winter, to examine the effects of temperature and growing stage on total mycorrhizal colonization. Examined rates of dormancy may also influence mychorrizal relationships and should be observed. Further investigation of mychorrizal relationships in *C. candidum*, may lead to more suitable cultivation practices to conserve these amazing plants. Examining the fungal presence of *C. candidum* may also serve as a profile for important soil microbes that heavily influence ecosystem biodiversity and productivity (van der Heijden et. al 1998).

Acknowledgements

I would like to thank the National Science Foundation and the Chicago Botanic Garden for giving me the opportunity to learn and participate in amazing plant science research. I also thank my mentor, Anne Nies, for her patience and guidance, throughout this internship and Dr. Louise Egerton for her guidance and allowing me to perform research in her lab.

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