

## The Advantages of being both Carnivorous and Mycorrhizal

### SUMMARY

Approximately 80% of the world's extant vascular plant families have members that participate in symbiotic relationships with arbuscular mycorrhizal (AM) fungi and this relationship is believed to have been prevalent amongst plants for more than 450 million years (Newman and Reddell 1987, Redecker et al. 2000). In fact, AM fungi likely played a critical role in the successful establishment of the first plants on land (Remy et al. 1994, Redecker et al. 2000). AM fungi confer a variety of benefits to the plant host in exchange for essential carbohydrates and vitamins. Such benefits to the plant may include improved resistance to disease, greater tolerance towards both biotic (e.g. nematodes) and abiotic stresses (e.g. drought), and improved nutrient acquisition (especially phosphorus) (Johnson et al. 1997, Auge 2001, Pozo and Azcon-Aguilar 2007, Hoeksema et al. 2010). For both the AM fungi and the plant hosts involved, the relationship is often essential for survival and competition.

Although the relationship between AM fungi and upland plants has been well known to science for quite some time, associations between AM fungi and wetland plants were for a long time thought to be non-existent. Recent research, however, has found that despite their aerobic nature, AM fungi are quite common in many oxygen limited wetlands (Ragupathy et al. 1990; Turner and Friese 1998). Furthermore, new studies are suggesting that carnivorous plants (most of which are obligate wetland species (Brewer et al. 2011)) may be mycorrhizal as well (Quilliam and Jones 2010; Quilliam and Jones 2012; Harikumar 2013; Abbott and Brewer, unpublished data). These recent findings are particularly surprising because it had been assumed that since carnivorous plants acquire the majority of their nutrients through the digestion of prey material, a relationship with AM fungi would be redundant (Juniper et al. 1989; Adlassnig et al. 2005; Brundrett 2009). The question thus remains: why be both carnivorous and mycorrhizal? In this study I propose that rather than being a relationship that is redundant with carnivory, AM fungi help carnivorous plants to obtain nutrients that cannot be gained solely via prey consumption.

Prey and soil likely differ with respect to the type of limiting nutrients each offers a carnivorous plant. In fire-prone pitcher plant bogs of the southeastern United States, carnivorous plants increase investment in carnivory (i.e., produce relatively larger traps) following fires (Brewer 1999, 2003), despite the fact that some important limiting nutrients are made more available in ash following fire (Brewer, unpublished data). These responses suggest that the types of limiting nutrients that are added to the soil in ash are not the same as those available from prey. Whereas phosphorus, potassium, magnesium, and manganese are added to the soil as ash, nitrogen is not (Brewer, unpublished data). Taken together, these responses suggest that prey are an important source of nitrogen, but may not be an important source of other nutrients. Otherwise, why would investment in carnivory increase at the same time the availability of these other nutrients (e.g., P, K, Mg, and Mn) increased (Givnish et al. 1984)? AM fungi therefore may be important for enabling carnivorous plants to access nutrients in the soil that are not readily available from prey. If so, the benefits of AM fungi might diminish following fire as the availability of nutrients other than nitrogen (e.g., P, K, Mg, and Mn) increase with ash addition.

Another factor that must be taken into consideration, though, is light. If light is limiting, investment in AM fungi might actually decrease because of the photosynthetic cost of feeding the fungi. Capogna et al. (2009), for instance, found that mycorrhizal colonization was positively

correlated with increased photosynthesis and even phosphorus after fire. A positive feedback loop may ensue when light and certain nutrients are no longer limiting after fire. For instance, greater light availability could increase the rate of photosynthesis, which could allow for a greater supply of carbon to the AM fungi, which in turn may maximize uptake of certain temporarily available nutrients that are directly or indirectly beneficial for photosynthesis (e.g., P, K, Mg, and Mn; Capogna et al. 2009). For carnivorous plants, it may be in their best interest to maximize photosynthesis with the assistance of AM fungi after fire so that they can rapidly produce large traps to overcome increased competition for nitrogen.

The goal of this study is to address three questions: (1) Do AM fungi colonize carnivorous plant roots more readily when P, K, Mg, and Mn are more limiting to plant growth than is N, during years without fire? (2) Do carnivorous plants invest more in carnivory when N is more limiting to growth, immediately after a fire? (3) Do investment in carnivory and root colonization by AM fungi both increase in response to reduced light limitation of growth, immediately after a fire? These questions will be tested by comparing mycorrhizal pale pitcher plants (*Sarracenia alata*) in a field experiment where I factorially manipulate two important aspects of fire: vegetation removal and nutrient (N vs. P, K, Mg, and Mn) addition.

## METHODS

*Location*—This study will be conducted in the coastal pitcher plant bogs within Grand Bay National Estuarine Research Reserve (30°27' N, 88°25' W). This reserve is located within Jackson County, MS and contains some of the largest intact tracts of pitcher plant bogs in the Gulf Coastal plain. This site was historically maintained by natural fire and has been relatively unimpacted by anthropogenic disturbance (Hilbert 2006).

*Experimental Setup*—To determine whether fire stimulated changes in soil nutrient availability, light availability, or a combination of the two are most important in regulating root colonization by AM fungi in carnivorous plants, I will compare *S. alata* in a field experiment where I factorially manipulate aboveground vegetation removal and nutrient inputs that might be expected from ash deposition. To accomplish such an experiment, this spring I will establish sixty 1 m<sup>2</sup> plots, each around a *S. alata* ramet, in a pitcher plant bog. I will then randomly assign half of the plots to vegetation removal treatments and the other half will be left intact. Within each of these treatments, I will randomly assign each plot to receive either N fertilizer, P, K, Mg, Mn fertilizer, or no fertilizer. The plots that receive no fertilizer will be sprayed with water to control for watering effect. Each treatment combination will have a sample size of ten. This experimental setup will allow me to see if (1) increased P, K, Mg, and Mn results in lower AM fungi production but not trap production; (2) N addition results in lower trap production but not lower AM fungi production or colonization; and (3) increased light availability interacts with one or both of the fertilizer treatments to either increase or decrease AM fungi production. At the end of the growing season, pitcher trap dimensional measurements will be taken (Ellison and Gotelli 2002) and the plants will be excavated so that root colonization rates can be assessed (Brundrett et al. 1996). Because soil moisture can also significantly influence AM fungi colonization, soil moisture measurements will be taken and added as a covariate within the data analyses (see below).

*Data Analysis*—This experiment will be a full factorial design and the data (level of root colonization and pitcher dimensions) will be analyzed using two-way ANCOVA (i.e., vegetation removal x fertilizer application) with percent moisture included as a covariate.

*Expected Results and Outcome*—I hypothesize that (1) AM fungi colonize carnivorous plant roots more readily when P, K, Mg, and Mn are more limiting to plant growth than is N, during years without fire; (2) carnivorous plants invest more in carnivory when N is more limiting to growth, immediately after a fire; (3) investment in carnivory and root colonization by AM fungi both increase in response to reduced light limitation of growth, immediately after a fire.

A significant main effect of nutrient treatment such that the addition of P, K, Mg, and Mn, but not N, reduces root colonization by AM mycorrhizae would support the hypothesis that AM fungi are most beneficial to carnivorous plants when P, K, Mg, and Mn are most limiting to growth (during periods without fire). A significant main effect of nutrient treatment such that the addition of N, but not P, K, Mg, and Mn, reduces production of carnivorous traps would support the hypothesis that carnivory is most beneficial to carnivorous plants when N is most limiting to growth (immediately after a fire). Third, a significant main effect of aboveground vegetation removal and nutrient treatment such that both AM root colonization and investment in carnivory increase most when aboveground vegetation is removed, irrespective of nutrient treatment, would support the hypothesis that AM mycorrhizae and carnivory are most beneficial to growth when light does not limit growth (immediately after a fire).

### **SIGNIFICANCE AND RELEVANCE**

This study will demonstrate how carnivorous plants can be used to get at more general questions about which nutrients are AM fungi most important for capturing. Such a study has parallels with studies of the benefits of AM fungi in plants with N-fixing symbionts. Other benefits include a greater understanding of whether fire regulated changes in light availability or nutrient availability are most important for regulating AM fungi colonization. This study would also improve our understanding of the ecology of AM fungi in wetlands. Recent research has suggested that the relationship between plants and AM fungi in wetlands is complicated due to frequent fluctuations in oxygen levels, phosphorus levels, and plant need for nutrients (Bohrer et al. 2004). However, unlike more productive wetlands within which soils often reach near anoxic conditions, redox levels in pitcher plant bogs are more moderate (e.g., the Grand Bay bogs maintained an average redox potential of +137.4 mV during the summer of 2014 (Abbott, pers. obs.)); thus, I expect that nutrients and plant requirements for nutrients, not oxygen, are the limiting factors for AM fungi in low nutrient bogs. By factorially manipulating nitrogen, phosphorus, and plant requirements for nutrients (i.e., by clipping leaves and forcing the plants to regenerate), I expect to see that AM fungi colonization is consistently higher in resprouting plants when the availability of nitrogen is high and phosphorus is low. To my knowledge, this will be the first mycorrhizal study that manipulates these factors separately in a nutrient poor wetland.

With only 3% of their original extent remaining today, Gulf Coast pitcher plant bogs are some of the most endangered wetland ecosystems in North America. Such a high degree of habitat loss—in addition to over-collection—has resulted in dramatic declines in carnivorous plant populations. It is therefore imperative that we gain a greater understanding of ecology of carnivorous plants and their habitat so that we adequately manage what we have left and restore

what is still salvageable. If this study reveals that the association between AM fungi and carnivorous plants is tightly related to resource availability, then that would provide support for the idea that such an association is not only beneficial, but it may be a requirement. This would thus provide an impetus for including mycorrhizae additions in the restoration of coastal pitcher plant bogs.

### **PLANNED USE OF FUNDS AND DISSEMINATION OF INFORMATION**

If funded, the money from the grant will be used to pay for the following:

- Five trips to the research site during growing season (\$1,765.00 (3,530 total miles at \$0.50 per mile))
- Dorm fee for myself and two helpers at the Grand Bay NERR field station (\$450.00 for three people staying three nights per trip (\$10.00 per person, per night))

Information garnered from this study will be disseminated to fellow researchers and managers via oral presentations that I give at national (e.g. Ecological Society of America) and/or international (e.g. Society of Wetland Scientists) research conferences, and through publication in an appropriate peer reviewed journal. I will also reach out to the more general public by presenting my findings at some of the many workshops and education programs offered by Grand Bay NERR. With the large variety of workshops and education programs offered at Grand Bay NERR, I can reach out to local residents ranging from private landowners to grade school students. My ultimate goal is to demonstrate to all Gulf Coast residents the importance and complexity of these pitcher plant bogs residing in their backyards.

### **REFERENCES CITED**

- Adlassnig, W., M. Peroutka, H. Lambers, and I. K. Lichtscheidl. 2005. The roots of carnivorous plants. *Plant and Soil* 274: 127-140.
- Auge, R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11:3-42.
- Brewer, J. S. 1999. Effects of fire, competition, and soil disturbances on regeneration of a carnivorous plant, *Drosera capillaris*. *American Midland Naturalist* 141: 28-42.
- Brewer, J. S. 2003. Why don't carnivorous pitcher plant's compete with non-carnivorous plants for nutrients? *Ecology* 84:451-462.
- Brewer, J.S., D.J. Baker, A.S. Nero, A.L. Patterson, R.S. Roberts, L.M Turner. 2011. Carnivory in plants as a beneficial trait in wetlands. *Aquatic Botany* 94:62-70.
- Brundrett, M.C., N. Bougher, B. Dell, T. Grove, and N. Malajczuk. 1996. Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research.
- Brundrett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* 320:37-77.
- Bohrer, K.E., C.F. Friese, and J.P. Amon. 2004. Seasonal dynamics of arbuscular mycorrhizal fungi in differing wetland habitats. *Mycorrhiza* 14:329-337.

- Capogna, F., A.M. Persiani, O. Maggi, G. Dowgiallo, G. Puppi, and F. Manes. 2009. Plant Ecology 204: 155-171.
- Ellison, A. M., and N. J. Gotelli. 2002. Nitrogen availability alters the expression of carnivory in the northern pitcher plant, *Sarracenia purpurea*. Proceedings of the National Academy of Sciences of the United States of America 99:4409-4412.
- Givnish, T. J., E. L. Burkhardt, R. E. Happel, and J. D. Weintraub. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost-benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. American Naturalist 124:479-497.
- Harikumar, V. S. 2013. Are there arbuscular mycorrhizal associations in carnivorous plants *Drosera burmanii* and *D. indica*? Botanica Serbica 37:13-19.
- Hilbert, K. W. 2006. Land cover change within the Grand Bay National Estuarine Research Reserve: 1974-2001. Journal of Coastal Research 22:1552-1557.
- Hoeksema, J.D., V.B. Chaudhary, C.A. Gehring, N.C. Johnson, J. Karst, R.T. Koide, A. Bringle, C. Zabinski, J.D. Bever, J.C. Moore, G.W.T. Wilson, J.N. Klironomos, and J. Umbanhowar. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13:394-407.
- Johnson, N. C., J. H. Graham, and F. A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist 135:575-586.
- Juniper, B. E., R. J. Robins, and D. M. Joel. 1989. The carnivorous plants. Academic Press.
- Newman, E. I., and P. Reddell. 1987. The distribution of mycorrhizas among families of vascular plants. New Phytologist 106:745-751.
- Pozo, M. J., and C. Azcon-Aguilar. 2007. Unraveling mycorrhiza-induced resistance. Current Opinion in Plant Biology 10:393-398.
- Quilliam, R. S., and D. L. Jones. 2010. Fungal root endophytes of the carnivorous plant *Drosera rotundifolia*. Mycorrhiza 20:341-348.
- Quilliam, R. S., and D. L. Jones. 2012. Evidence for host-specificity of culturable fungal root endophytes from the carnivorous plant *Pinguicula vulgaris* (Common Butterwort). Mycological Progress 11.
- Ragupathy, S., V. Mohankumar, and A. Mahadevan. 1990. Occurrence of vesicular-arbuscular mycorrhizae in tropical hydrophytes. Aquatic Botany 36:287-291.
- Redecker, D., R. Kodner, and L. E. Graham. 2000. Glomalean fungi from the Ordovician. Science 289:1920-1921.
- Remy, W., T. N. Taylor, H. Hass, and H. Kerp. 1994. 4-hundred-million-year-old vesicular-arbuscular mycorrhizae. Proceedings of the National Academy of Sciences of the United States of America 91:11841-11843.
- Turner, S.T., and C.F. Friese. 1998. Plant-mycorrhizal community dynamics associated with a moisture gradient within a rehabilitated prairie fen. Restoration Ecology 6:44-51.