E. Kathryn Barto
barto.3@wright.edu
Wright State University
Dayton, Ohio

Native plant and mycorrhizae establishment after garlic mustard removal: Implications for restoration

2006 EPA Graduate Fellowship Conference — From Discovery to Solutions: Generation Y Scientists Lead The Way

INTRODUCTION

Garlic mustard (*Alliaria petiolata*,GM) is an invasive plant in North America that produces many noxious compounds (glucosinolates, flavonoid glycosides, alliarinoside) and is likely allelopathic[1-4]. Arbuscular mycorrhizae (AM) are fungi that associate with ~80% of woodland plants. They are fed by the plant, act as an extended root system for the plant, and can only survive while associated with a plant. Pale jewelweed (*Impatiens pallida*) is native to North America, grows in the same habitat that garlic mustard invades, and relies on AM for "normal" growth. GM cannot associate with AM and actually inhibit growth of AM fungi[2,4]

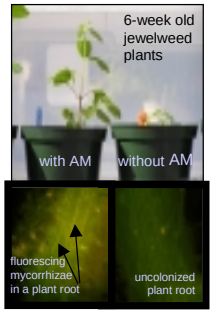
We tested the effects of three ways of removing GM on the establishment of pale jewelweed and its associated AM.

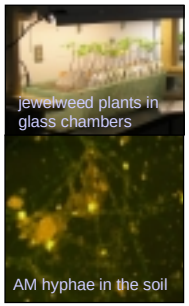
METHODS - planting

We made root viewing chambers by sandwiching two 13 x 30cm glass plates together with silicon[5]. We grew GM in these chambers for 4 months in two types of non-sterile field soil: field soil mix, and field soil mix and activated carbon to absorb organic compounds[1]. We removed GM plants by: painting the leaves with RoundUp™ (Monsanto) which left the entire root system to die quickly, cutting and removing the shoot only which left the entire root system to die slowly, or by pulling out as much of the plant as possible, which left only small amounts of GM root tissue to die quickly.

We added AM inocula to half the chambers in each treatment group and planted a newly germinated jewelweed seed.

There were five chambers in each treatment group.





METHODS – monitoring

Every week we monitored plant height, and AM in the root system using epifluorescence microscopy along five transect lines (location shown at right)[5]. We calculated a Root Colonization Index (RCI) by estimating percent colonization of roots as 0%, 1-25%, 26-50%, 51-75%, 76-100% and calculating the percentage of roots observed in each category as (P0, P25, P50, P75, P100).

$$RCI = 4(P100) + 3(P75) + 2(P50) + (P25)$$

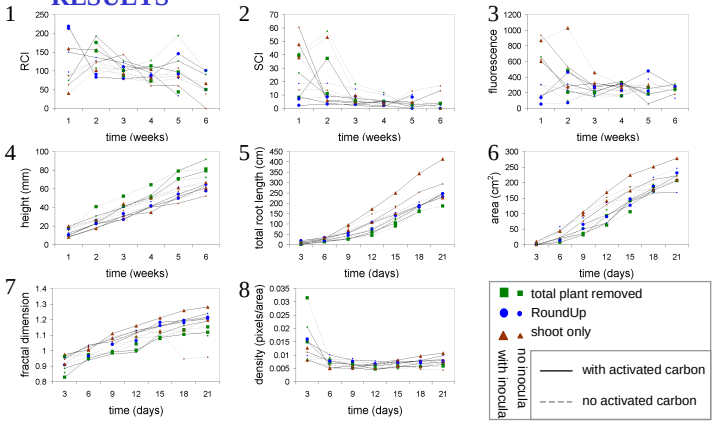
We calculated a Soil Colonization Index (SCI) by estimating the number of hyphae in soil as 0, 1-3, 4-6, 7-9, =10 and calculating the percentage of hyphae observed in each category as (P0, P3, P6, P9, P10).

$$SCI = 4(P10) + 3(P9) + 2(P6) + (P3)$$

Every three days we traced plant roots and determined: total root length, area of root system, density of roots in root system (pixels/area), and box-counting fractal dimension (a measure of the exploration efficiency of the system).

All endpoints were analyzed using ANOVA with activated carbon, inocula, and removal method as factors.

RESULTS



Figures 1: Root colonization index for mycorrhizae in jewelweed roots (estimates **quantity** of AM within plant roots), **2:** Soil colonization index for mycorrhizae in soil around jewelweed roots, **3:** Fluorescence of jewelweed root segments (estimates **quality** of AM within plant roots), **4:** Height of jewelweed plants, **5:** Total root length of jewelweed plants, **6:** Total root area of jewelweed plants, **7:** Box-counting fractal dimension of jewelweed root systems, **8:** Density of jewelweed root systems (pixels/area). N=2-5.

DISCUSSION

Jewelweed plants and their mycorrhizae did best with a minimum of dead GM root tissue left in the soil.

The quantity of AM inside plant roots was unaffected by treatments (Figure1). When the entire GM plant was removed, AM were healthier (Figures 2 and 3, SCI: Week 1: $F_{2,31}$=7.56, p=0.0021; Week 2: $F_{2,47}$=9.41, p=0.0004; Week 4: $F_{2,48}$=3.33, p=0.0442; Week 6: $F_{2,35}$=6.76, p=0.0033; fluorescence: Week 1: $F_{2,21}$=11.14, p=0.0005; Week 2: $F_{1,44}$=4.39, p=0.0420). In these chambers, jewelweed root systems were smaller, denser, and less efficient at soil exploration (Figures 5-8, root system area: Day 24: $F_{2,11}$=3.94, p=0.0513; fractal dimension: Day 18: $F_{2,35}$=2.54, p=0.0933; Day 21: $F_{2,26}$=2.77, p=0.0814; density: Day 12: $F_{2,47}$=6.27, p=0.0039; Day 15: $F_{2,44}$=7.47, p=0.0016; Day 18: $F_{2,35}$=3.15, p=0.0567; Day 24: $F_{2,11}$=7.66, p=0.0082), meaning that plants did not invest in large, efficient root systems because AM were providing them with water and nutrients[6]. These jewelweed plants had extra energy to invest in above ground growth and were taller than plants in other chambers at the end of the experiment (Figure 4, height: Week 4: F2,5=4.39, p=0.0793; Week 5: F2,38=8.70, p=0.0008; Week 6: F2,41=10.11, p=0.0003). Taller jewelweed plants produce larger seed, which produce larger plants in the next generation[7-8], so jewelweed plants not exposed to large amounts of dead GM root tissue should produce healthier offspring than plants that are exposed to dead GM tissue.

Dead root tissue, especially of allelopathic species, can significantly inhibit growth of native plants and their mycorrhizae, even if they are introduced after the offending plant has been killed.

ACKNOWLEDGEMENTS

I'd like to thank my advisor, Don Cipollini, committee members James Amon, Wayne Carmichael, Carl Friese, and Audrey McGowin, also Eusondia Barto, Steph Enright, Jeanne Mbagwu, Karyn Pinson and Cherissa Rainey for help in the lab.

REFERENCES

1. Prati D, O Bossdorf (2004) Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). *American Journal of Botany* 91: 285-288.
2. Roberts KJ, RC Anderson (2001) Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *American Midland Naturalist* 146: 146-152.
3. Vaughn SF, MA Berhow (1999) Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). *Journal of Chemical Ecology* 25: 2495-2504.
4. Stinson KA, SA Campbell, JR Powell, BE Wolfe, RM Callaway, GC Thelen, SG Hallett, D Prati, JN Klironomos (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLOS Biology* 4: 727-731.
5. Friese CF, MF Allen (1991) The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. *Mycologia* 83: 409-418.
6. Oliver AJ, SE Smith, DJD Nicholas, W Wallace (1983) Activity of nitrate reductase in *Trifolium subterraneum*; effects of mycorrhizal infection and phosphate nutrition. *New Phytologist* 94: 63-79.
7. Waller DM (1984) Difference in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. *Evolution* 38: 427-440.
8. Waller DM (1982) Factors influencing seed weight in *Impatines capensis* (Balsaminaceae). *American Journal of Botany* 69: 1470-1475.

This fellow is sponsored by EPA's STAR or Greater Research Opportunities (GRO) Program.