

Competitive Effects of Increasing Densities of Non-Native *Microstegium vimineum* (Japanese stiltgrass) on *Panax quinquefolius* (American ginseng) growth and

Background

Panax quinquefolius L. (American ginseng) is a rare but highly important herb that is native to the eastern United States. It is extremely valuable because of the medicinal properties of ginsenosides, secondary compounds found in the roots and shoots of the plant (Searels et al. 2013). However, populations of *P. quinquefolius* in the southern Appalachians are becoming increasingly threatened, mainly because of overharvesting and a resulting decline in genetic diversity within populations. Other threats to *P. quinquefolius* populations include climate change, disturbance, deer browse, and competition with co-occurring plant species (McGraw et al. 2013).

Competitive interactions between *P. quinquefolius* and *Alliaria petiolata* (M. Bieb) Cavara & Grande (garlic mustard), an invasive allelopathic competitor of ginseng, were shown to increase *P. quinquefolius* mortality (Wixtead and McGraw 2010). *Microstegium vimineum* (Japanese stiltgrass) is another invasive species that often co-occurs with *P. quinquefolius* (Wixtead and McGraw 2009) in shady southeastern forests. *Microstegium vimineum* is particularly interesting because of its ability to alter soil fertility (Kourtev et al. 1998) which might affect ginsenoside production (Li and Mazza 1999), its highly dense growth, and its ability to flourish in low-light environments (Cheplick and Fox 2011).

Because *M. vimineum* is a highly invasive species that may co-occur with *P. quinquefolius* in shady southeastern forests, it could potentially be harmful to threatened ginseng populations. This study aims to examine the potential effects of increasing *M. vimineum* densities on populations of *P. quinquefolius*. I will examine survivorship and biomass of ginseng seedlings as well as any potential effects on soil quality and/or ginsenoside production. I expect that increasing densities of *M. vimineum* will have greater competitive effects resulting in slower growth, lower photosynthetic rates, higher mortality, and reduced ginsenoside content.

Methods

This study will use a full additive design, with varying densities of *Microstegium vimineum* planted in 11 cm diameter pots with a single seedling of *P. quinquefolius*. We will use 5 *M. vimineum* densities (0, 105.3, 526.3, 947.4, and 1368.4 plants per m², after Cheplick and Fox 2011), spanning the range of its natural densities in the wild, with 20 replicates per treatment. Pots will be filled with soil collected from field sites where *P. quinquefolius* occurs naturally. Seedlings of *P. quinquefolius* will be germinated from stratified seed purchased from a commercial seed producer. These will be transplanted into native soil and allowed to recover from transplant shock. Pots will be randomly assigned to one of the five density treatments and field collected *M. vimineum* will be transplanted at appropriate densities. While planting, soils from a subset of 25 pots (five randomly selected from each density treatment) will be collected and analyzed for soil nutrient availability. Soil from these same pots will be analyzed again at the end of the experiment to determine if *M. vimineum* altered nutrient availability during the experiment. Once pots have been prepared, they will be placed outside under natural shade and allowed to grow for twelve weeks. Growth (height and leaf area) and survivorship will be censused weekly during the study. Photosynthetic rates will be measured at weeks 6 and 12. At

the end of the growing period, roots and shoots will be collected, dried, and weighed for total biomass. We will also analyze ginsenoside concentrations in the roots using standard extraction practices and High Performance Liquid Chromatography (Court et al. 1996). Survivorship will be compared among treatments with repeated measures Analysis of Variance (ANOVA). Growth data will be used to calculate relative growth rate (RGR). Root, shoot, and total biomass, RGR, soil nutrient availability at harvest and ginsenoside concentration will be compared among treatments with ANOVA and post-hoc means comparison.

Budget

Stipend for 12 weeks of work	\$1500
Soil analysis (5 pots per treatment [5], before and after; \$5 each). (NCDA&CS)	\$250
Planting supplies, including pots (Jesse Israel)	\$100
Stratified ginseng seeds (Wildgrown.com)	\$50
Coin envelopes for drying plant samples after harvest (Staples)	\$30
Consumables for ginsenoside analysis (Fisher Scientific)	\$570
Total	\$2500

Budget Justification

I request a stipend (\$1500) to support myself while working on this research project. I request funding to purchase stratified ginseng seeds from a commercial producer and planting supplies for planting these in pots. Japanese grass seedlings will be harvested from invaded areas on campus. Soils will be analyzed at the North Carolina Department of Agriculture and Consumer Services laboratory in Raleigh, NC. Coin envelopes will be used to store samples while drying and for storage after. Consumables for extraction and HPLC analysis (methanol, acetonitrile, etc.) will be purchased to supplement supplies already in the lab. HPLC analysis will be conducted with the assistance of Dr. Jeff Wilcox in the Environmental Studies Department.

Timeline of Research Activities

May 2015: Plant *M. vimineum* and *P. quinquefolius* in pots, initial soil nutrient analysis

June-August 2015: Growing period. Monitor growth, survival, and photosynthetic rates

August 2015: Harvest *P. quinquefolius* roots and shoots

Fall 2015: Soil and ginsenoside analyses

Spring 2016: Analyze data, prepare manuscript for publication in UNCA journal and presentation for UNCA Symposium, Association of Southeastern Biologist, and other regional meetings.

References

Cheplick, G. P. and J. Fox. 2011. Density-dependent growth and reproduction of *Microstegium vimineum* in contrasting light environments. *Journal of the Torrey Botanical Society* 138:62-72.

- Court, W.A., Hendel, J.G., & Elmi, J. (1996). Reversed-phase high-performance liquid chromatographic determination of ginsenosides of *Panax quinquefolius*. *Journal of Chromatography A*. 755: 11-17.
- Kourtev, P. S., J. G. Ehrenfeld, and W. Z. Huang. 1998. Effects of exotic plant species on soil properties in hardwood forests of New Jersey. *Water, Air, and Soil Pollution* 105: 493-501.
- Li, T.S.C. and G. Mazza. 1999. Correlations between leaf and soil mineral concentrations and ginsenoside contents in American ginseng. *HortScience* 34:85-87.
- McGraw, J. B., A. E. Lubers, M. Van der Voort, E. H. Mooney, M. A. Furedi, S. Souther, J. B. Turner, and J. Chandler. 2013. Ecology and conservation of ginseng (*Panax quinquefolius*) in a changing world. *Annals of the New York Academy of Sciences* 1286:62-91.
- Searels, J. M., K. D. Keen, J. L. Horton, H. D. Clarke, and J. R. Ward. 2013. Comparing ginsenoside production in leaves and roots of wild American Ginseng (*Panax quinquefolius*). *American Journal of Plant Sciences* 4:1252-1259.
- Wixtead K. and J. B. McGraw. 2009. A *Panax*-centric view of invasive species. *Biological Invasions* 11:883-893.
- Wixtead, K. L. and J. B. McGraw. 2010. Competitive and allelopathic effects of garlic mustard (*Alliaria petiolata*) on American ginseng (*Panax quinquefolius*). *Plant Ecology* 208:347-357.