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

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Abstract

Panax quinquefolius L. (American ginseng) is a rare but commercially important herb found in shady forests in eastern North America. In addition to the negative effects of overharvesting (decreasing population size and loss of genetic diversity, among others), *P. quinquefolius* faces threats from invasive species such as *Microstegium vimineum* (Trin.) A. Camus (Japanese stiltgrass), a non-native grass that can grow in high densities in low-light environments. The aim of this study was to examine potential competitive effects of increasing *M. vimineum* densities, focusing on seedling mortality and growth rates of *P. quinquefolius* seedlings. Commercially-grown *P. quinquefolius* seedlings were planted in pots with five different densities of *M. vimineum*, and were censused once per week. Soil samples were collected and analyzed before and after treatments. After 12 weeks, the plants were harvested and dried, biomass of each species was weighed, and *P. quinquefolius* leaf area was measured. Although *P. quinquefolius* root and shoot biomass were significantly higher in low-density treatments, there were no significant differences between high-density treatments and control pots. In addition, survivorship decreased in high-density treatments. Soil nutrients also showed significant differences between pre- and post-treatment analysis, indicating soil-altering abilities in *M. vimineum*. These data point to a possible competitive effect of *M. vimineum* on *P. quinquefolius*, though more data and larger sample sizes are needed to confirm this relationship.

1. Introduction

Panax quinquefolius L. (American ginseng) is a rare, economically important herb that is native to the eastern United States. It is extremely valuable because of the medicinal properties of ginsenosides, secondary compounds which are found in the leaves and roots of the plant[1,2]. However, populations of *P. quinquefolius* in the southern Appalachians are becoming increasingly threatened, mainly due to overharvesting and a resulting decline in genetic diversity within unprotected populations[3]. This decreased genetic diversity leaves *P. quinquefolius* more vulnerable to other threats including climate change, disturbance, deer browse, and competition with co-occurring plant species[4]. This last threat was examined in a 2010 study by Wixted and McGraw[5], which showed that competitive interactions between *P. quinquefolius* and *Alliaria petiolata* (M. Bieb) Cavara & Grande (garlic mustard), an invasive allelopathic competitor of ginseng, increased *P. quinquefolius* mortality and decreased survivorship. Although certain *P. quinquefolius* populations show high levels of invasion[6], relatively few studies have examined the effects of competition between *P. quinquefolius* and invasive species.

Microstegium vimineum (Trin.) A. Camus (Japanese stiltgrass) is an invasive C_4 grass that has been shown to grow alongside *P. quinquefolius*[6]. It is particularly harmful due to its ability to change soil quality by elevating pH and changing soil nutrient cycling (for instance, by increasing concentrations of extractable NO_3)[7,8], as well as its highly dense growth and ability to flourish in low light environments[9]. Areas with higher levels of disturbance are more prone to *M. vimineum* invasions[10]. Although *P. quinquefolius* tends to occur in less disturbed areas[11], deer-mediated disturbance could play a role in increasing invasive species abundance[12]. Because deer frequently feed on ginseng, this could potentially facilitate invasion of *M. vimineum* into populations of *P. quinquefolius*, increasing the chance that the two species will co-occur. Although *M. vimineum* is not allelopathic, it can alter soil quality, and thus it may have a negative effect on *P. quinquefolius* survivorship and ginsenoside production. 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.

The aim of this study was to examine the potential effects of increasing *M. vimineum* densities on growth and survival of *P. quinquefolius*. I looked at survivorship and biomass of ginseng seedlings as well as potential effects on soil quality, expecting that increasing densities of *M. vimineum* would have greater competitive effects resulting in reduced growth and higher mortality of *P. quinquefolius* seedlings.

2. Methods

I obtained *P. quinquefolius* seeds from wildgrown.com and harvested *M. vimineum* seedlings from invaded areas on the UNC Asheville campus. Both species were allowed to germinate and grow in growth chambers (25ºC, 75% RH, 14/10 light dark cycle with 500 μmol m^{-2} s^{-1} of light). In May 2015, I planted *M. vimineum* seedlings in 11 cm diameter pots, filled with soil collected from field sites where *P. quinquefolius* occurs naturally, each with a single *P. quinquefolius* seedling. Five different densities of *M. vimineum* were used, spanning its natural range of densities[9]: 0, 105.3, 526.3, 947.4, and 1368.4 plants/m^2. This translates into 0, 2, 10, 19, and 27 seedlings per pot, respectively. I used 6 replicates of each treatment, resulting in a total of 30 pots. While planting, soils from a subset of 10 pots (two randomly selected from each density treatment) were collected and analyzed for soil nutrient availability by the North Carolina Department of Agriculture and Crop Science.

All pots were placed in a shaded field in Asheville, NC from May 20 to August 11, 2015 under a canopy of *Acer rubrum*. After the first week, plants that did not survive due to transplanting stress were replaced. The plants were watered once daily, and censused roughly once per week. In early August 2015, all *P. quinquefolius* and *M. vimineum* plants were harvested, and leaf area of each surviving *P. quinquefolius* seedling was measured using ImageJ software (National Institutes of Health, Bethesda, MD). The roots and shoots of *P. quinquefolius* were separated and placed in individual coin envelopes, and all *M. vimineum* plant material in each pot was placed in individual envelopes. Plant material was dried for 48 hrs at 60° C in a drying oven, and each sample (each *P. quinquefolius* root and shoot separately, and all *M. vimineum* plant material per pot) was weighed for total biomass. After harvesting, soils from a subset of 15 pots (three randomly selected from each density treatment) were collected and analyzed for soil nutrient availability.

2.1. Statistical Analyses

Total *P. quinquefolius* biomass per treatment, mean *P. quinquefolius* root and shoot biomass, and average *P. quinquefolius* leaf area per treatment were compared using one-way analysis of variance (ANOVA) tests. Post-hoc Tukey's Studentized Range (HSD) tests were used to compare treatment means. In addition, separate regression analyses were performed for *P. quinquefolius* root and shoot biomass versus total *M. vimineum* biomass per treatment. All statistical analyses were conducted using SAS (Statistical Analysis Software, Cary, NC).

3. Results

Root biomass and shoot biomass of *P. quinquefolius* were significantly higher in the 105.3 plants/m^2 pots when compared to the higher density treatments (P = 0.0012 and P = 0.0011, respectively; Figs. 1A, 1B). However, the higher density *M. vimineum* treatments did not differ significantly from the control. The total *P. quinquefolius* biomass per treatment showed similar significant differences between the 105.3, 947.4, and 1368.4 plants/m^2 treatments (P = 0.0037; Fig. 1C). Mean *P. quinquefolius* leaf area was also significantly higher in the 105.3 plants/m^2 pots when compared to the higher density treatments (P = 0.0012; Fig. 2). Furthermore, a significant negative relationship was found between *P. quinquefolius* shoot biomass and total *M. vimineum* biomass per pot (P = 0.0042; Fig. 3). *P. quinquefolius* root biomass and total *M. vimineum* biomass per pot did not show a significant relationship (Fig. 4).

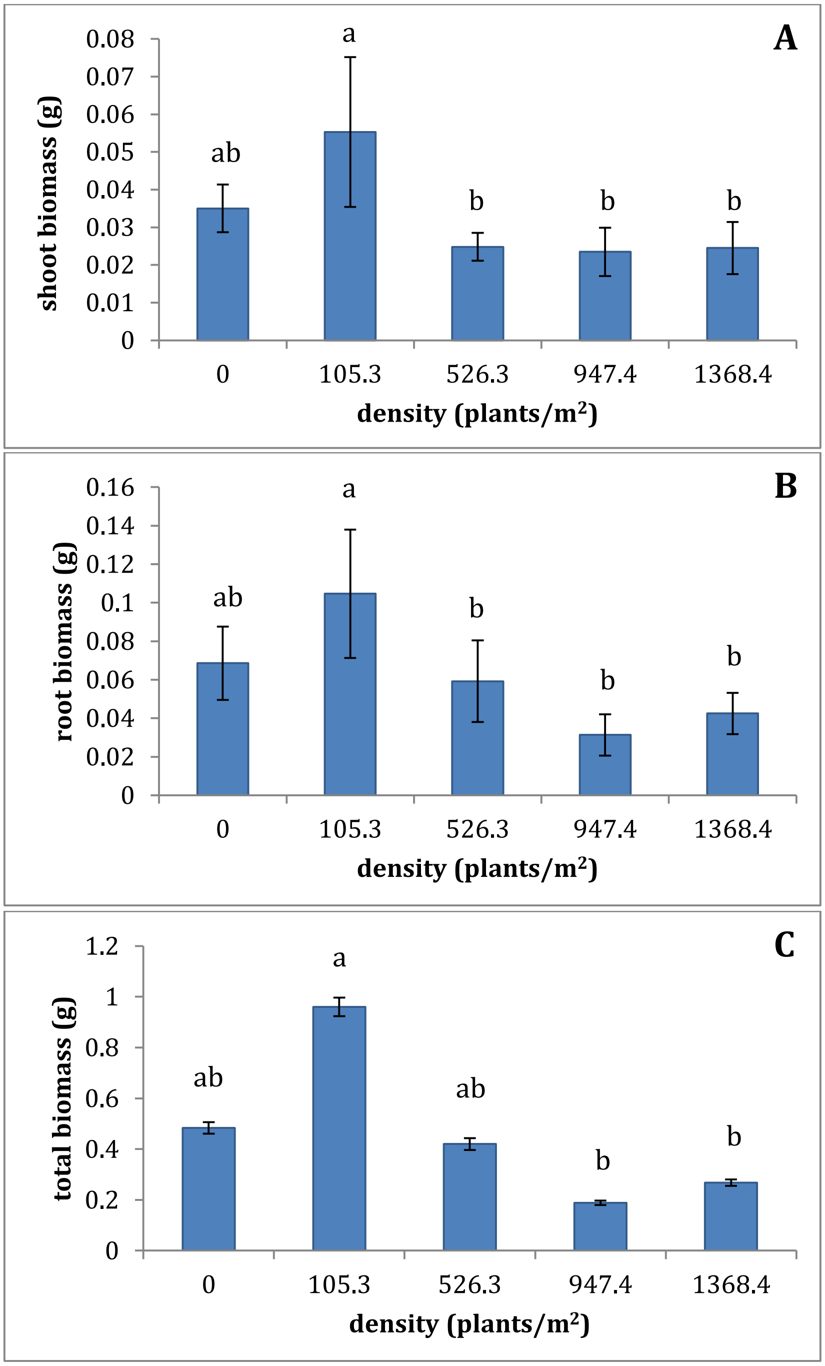

Figure 1. Mean *Panax quinquefolius* shoot biomass (A, $P = 0.0011$), root biomass (B, $P = 0.0012$), and total biomass (C, $P = 0.0037$) among *Microstegium vimineum* density treatments. Means with the same letter are not significantly different at $P < 0.05$ using a one-way ANOVA test.

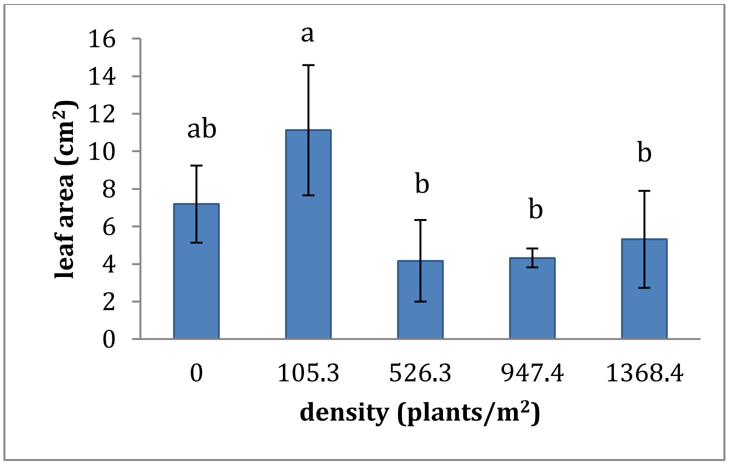

Figure 2. Mean leaf area of *Panax quinquefolius* seedlings among *Microstegium vimineum* density treatments (P = 0.0012). Means with the same letter are not significantly different at $P < 0.05$ using a one-way ANOVA test.

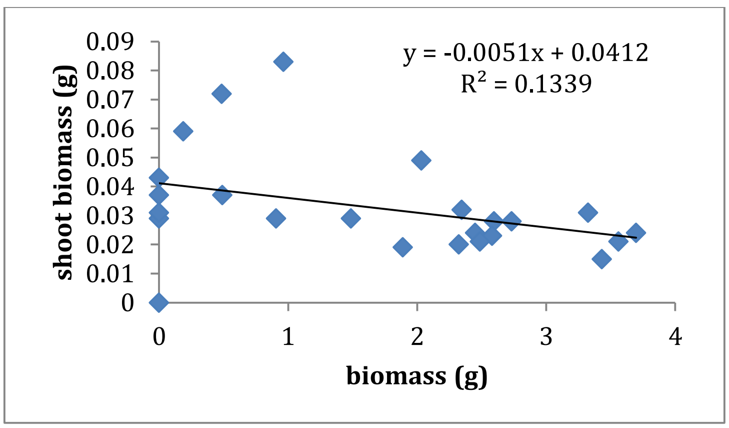

Figure 3. Regression analysis of *Panax quinquefolius* shoot biomass per treatment as a function of total *Microstegium vimineum* biomass per treatment (P = 0.0042).

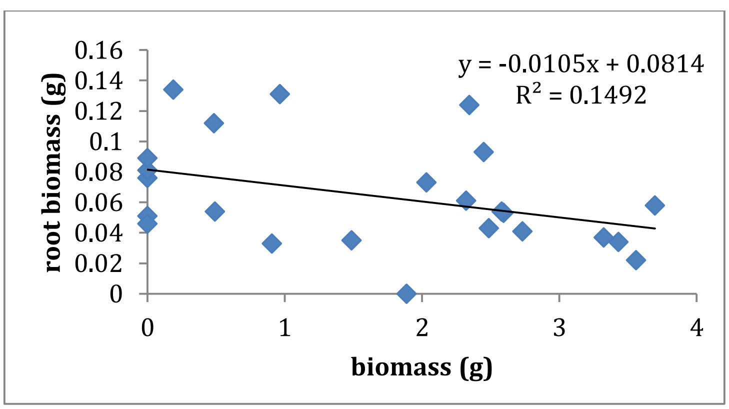

Figure 4. Regression analysis of *Panax quinquefolius* root biomass per treatment as a function of total *Microstegium vimineum* biomass per treatment (P = 0.0596).

Soil analyses showed significant differences between treatments in bulk density, cation exchange capacity (CEC), pH, potassium, calcium, magnesium, sulfur, manganese, and zinc levels (Table 1). Specifically, pre-treatment values for CEC, potassium, calcium, magnesium, sulfur, and zinc levels were significantly higher than all post-treatment values ($P < 0.0001$ for all values), while pH was significantly decreased in the pre-treatment soil ($P < 0.0001$). Pre-treatment bulk density was higher in the 0 and 105.3 plants/m^2 treatments ($P = 0.0008$), but did not differ significantly from the higher density treatments. Manganese levels in the 947.4 plants/m^2 treatment were significantly higher than all other treatments except the 0 plants/m^2 treatment ($P = 0.0019$).

Table 1. Mean (± SE) values for given soil parameters. Within each row, means with different superscript letters were significantly different at $P = 0.05$.

			Density (plants/m^2)			
	Pre-treatment	0	105.3	526.3	947.4	1368.4
HM (g/100cc)	0.55 ± 0.01 ^{a}	0.56 ± 0.03 ^{a}	0.53 ± 0.02 ^{a}	0.53 ± 0.02 ^{a}	0.57 ± 0.01 ^{a}	0.54 ± 0.02 ^{a}
W/V (g/cc)	0.95 ± 0.00 ^{a}	0.88 ± 0.01 ^{b}	0.88 ± 0.03 ^{b}	0.91 ± 0.01 ^{ab}	0.91 ± 0.02 ^{ab}	0.93 ± 0.01 ^{ab}
CEC (meq/100cc)	19.60 ± 0.24 ^{a}	14.70 ± 0.32 ^{b}	15.40 ± 0.29 ^{b}	15.23 ± 0.12 ^{b}	15.13 ± 0.43 ^{b}	14.93 ± 0.27 ^{b}
BS (%)	100.00 ± 0.00 ^{a}	100.00 ± 0.00 ^{a}	100.00 ± 0.00 ^{a}	100.00 ± 0.00 ^{a}	100.00 ± 0.00 ^{a}	100.00 ± 0.00 ^{a}
Ac (meq/100cc)	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
pH	7.02 ± 0.02 ^{a}	7.57 ± 0.03 ^{b}	7.70 ± 0.00 ^{b}	7.60 ± 0.00 ^{b}	7.63 ± 0.03 ^{b}	7.63 ± 0.03 ^{b}
P (mg/dm3)	74.30 ± 0.58 ^{a}	76.33 ± 2.40 ^{a}	70.67 ± 1.45 ^{a}	74.00 ± 1.15 ^{a}	73.67 ± 1.20 ^{a}	72.00 ± 1.73 ^{a}
K (mg/dm3)	445.80 ± 2.50 ^{a}	206.00 ± 4.51 ^{b}	234.67 ± 4.41 ^{b}	220.67 ± 12.68 ^{b}	221.33 ± 15.30 ^{b}	220.67 ± 10.65 ^{b}
Ca (mg/dm3)	3334.40 ± 45.91 ^{a}	2612.00 ± 58.65 ^{b}	2726.00 ± 52.25 ^{b}	2711.33 ± 17.46 ^{b}	2686.00 ± 86.19 ^{b}	2646.00 ± 54.50 ^{b}
Mg (mg/dm3)	219.40 ± 1.13 ^{a}	139.67 ± 3.76 ^{b}	147.00 ± 3.46 ^{b}	142.67 ± 2.33 ^{b}	141.33 ± 3.76 ^{b}	142.00 ± 0.58 ^{b}
S (mg/dm3)	247.10 ± 5.50 ^{a}	20.67 ± 0.67 ^{b}	25.33 ± 0.67 ^{b}	30.33 ± 2.85 ^{b}	28.67 ± 4.63 ^{b}	26.67 ± 3.84 ^{b}
Mn (mg/dm3)	32.85 ± 0.26 ^{b}	36.93 ± 3.12 ^{ab}	32.73 ± 1.66 ^{b}	34.73 ± 0.98 ^{b}	45.13 ± 5.14 ^{a}	33.53 ± 0.92 ^{b}
Zn (mg/dm3)	6.49 ± 0.04 ^{a}	5.63 ± 0.09 ^{b}	5.80 ± 0.12 ^{b}	5.93 ± 0.07 ^{b}	6.03 ± 0.27 ^{b}	5.87 ± 0.15 ^{b}
Cu (mg/dm3)	4.30 ± 0.40 ^{a}	3.63 ± 0.03 ^{a}	3.67 ± 0.07 ^{a}	3.77 ± 0.03 ^{a}	3.87 ± 0.09 ^{a}	3.77 ± 0.03 ^{a}
Na (meq/100cc)	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
K (meq/100cc)	1.14 ± 0.01 ^{a}	0.53 ± 0.01 ^{b}	0.60 ± 0.01 ^{b}	0.56 ± 0.03 ^{b}	0.57 ± 0.04 ^{b}	0.56 ± 0.03 ^{b}
Ca (meq/100cc)	16.68 ± 0.23 ^{a}	13.06 ± 0.29 ^{b}	13.63 ± 0.26 ^{b}	13.56 ± 0.09 ^{b}	13.43 ± 0.43 ^{b}	13.23 ± 0.27 ^{b}
Mg (meq/100cc)	1.81 ± 0.01 ^{a}	1.15 ± 0.03 ^{b}	1.21 ± 0.03 ^{b}	1.17 ± 0.02 ^{b}	1.16 ± 0.03 ^{b}	1.17 ± 0.01 ^{b}

4. Discussion

Although there were no significant differences between the control and the higher density treatments (526.3, 947.4, and 1368.4 plants/m^2) for any of the measured parameters, the difference between the 105.3 plants/m^2 treatment and the higher density treatments suggests a competitive effect of *M. vimineum* on *P. quinquefolius*. The relatively low success of the control plants when compared to the 105.3 plants/m^2 treatment was unexpected, though it is possible that plants growing at lower densities may be more prone to herbivory damage by specialist insects[13]. However, as this study did not quantify herbivory damage, the degree to which herbivory affected differences among treatments remains unclear.

Despite the fact that *P. quinquefolius* can grow in a wide range of soil conditions[11], the effects of *M. vimineum* on soil quality may have played a role in decreasing *P. quinquefolius* growth. These results align with previous studies[5], which found that *P. quinquefolius* was negatively affected by competition from native and invasive species as well as by allelopathy from invasive *Alliaria petiolata*. These data suggest that *P. quinquefolius* is a relatively weak competitor when compared with fast-growing invasives.

Because of varying levels of *M. vimineum* mortality, however, the regression analyses of total *M. vimineum* biomass per *P. quinquefolius* may yield more accurate results than simply comparing treatments. These data reveal a stronger negative correlation between *P. quinquefolius* shoot biomass and *M. vimineum* biomass than between *P. quinquefolius* root biomass and *M. vimineum* biomass. Although these data point to competition between *M. vimineum* and *P. quinquefolius*, the high mortality rates of both species and overall low sample sizes weaken this conclusion, meaning that further research will be needed to confirm this relationship. Furthermore, the determinate growth of *P. quinquefolius* in each growing season[14] placed limitations on this study; future studies should examine seedling growth over multiple growing seasons.

In addition, future research should aim to examine the effect of *M. vimineum* on ginsenoside production. Previous studies have shown that soil nutrient levels and fertility influence both *P. quinquefolius* root weight gain[15] and ginsenoside synthesis in roots and leaves[16, 17]. Although my study did not examine ginsenoside production, my results showed both changing soil nutrient levels caused by *M. vimineum* invasion and decreased *P. quinquefolius* root and shoot growth. Therefore, is likely that *M. vimineum* invasion would have a direct effect on ginsenoside production by reducing the amount of nutrients gained by *P. quinquefolius*. As this study did not examine secondary metabolite production, this would be a logical next step to take for future research.

5. Acknowledgements

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