IMPACTS OF AN INVASIVE GRASS *MICROSTEGIUM VIMINEUM* (TRIN.) A. CAMUS ON GROWTH AND SURVIVAL OF THE NATIVE HERB, *IMPATIENS CAPENSIS* MEERB.

A thesis presented to the faculty of the Graduate School of Western Carolina University in
partial fulfillment of the requirements for the degree of Master of Science in Biology.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

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Microstegium vimineum (Trin.) A. Camus is an invasive annual grass in the eastern United States. It can form dense monocultures along roads and forest understories, displacing native species due to its superior ability to compete for light and soil resources or indirect effects on the soil ecosystem. *Impatiens capensis* Meerb. is a native annual herb that grows in ditches, along creeks, and along road sides. *Microstegium* and *Impatiens* are annuals and can co-occur. Competition between *Microstegium* and *Impatiens* was examined in the field in two locations: Kentucky and North Carolina. A total of 180 plots (25cm×25cm) were established in populations where each species was growing by itself (single species plots) or growing together (mixed species plots). *Microstegium* density and stem mass were compared between the single-species and mixed-species plots in both locations. *Impatiens* height (cm), stem mass (g), number of fruits, and fruit mass (g) were compared between plot types and locations. I hypothesized that the invasive species would be the stronger competitor and would outcompete *Impatiens* by lowering its growth and survival. In addition, I compared soil carbon, nitrogen and C: N ratios from the single species and mixed species plots to examine potential indirect effects of *Microstegium* on the soil ecosystem. In contrast to my hypothesis, the native *Impatiens* outcompeted invasive *Microstegium* by reducing its growth and survival. Both mass and density of *Microstegium* were lower in plots where they grew with *Impatiens* than in plots where they grew alone. In addition, *Impatiens* plants were taller and had greater stem mass in mixed plots with *Microstegium*. Further, number of fruits and fruit mass of *Impatiens* did not differ significantly when plants were growing with *Microstegium.* There was no evidence of soil mediated indirect effects of either species. Soil carbon was higher in Kentucky. However, soil nitrogen and carbon did not differ between plot types in either Kentucky or North Carolina, suggesting the observed growth differences of *Microstegium* and *Impatiens* between plot types was not due to effects of these species on the soil ecosystem. The results of this study suggest that the invasive grass *Microstegium vimineum* has no negative impacts on growth and survival of the native herb *Impatiens capensis*.

INTRODUCTION

A growing number of invasive plant species are colonizing natural areas and threatening the diversity of native species (Hejda et al., 2009). Invasive species include both nonnative, or introduced species, and native species that spread outside their normal range. Almost half of the plant species in the United States that are at risk of extinction are endangered because of the effects of introduced species alone or in combination with other processes such as habitat loss (Gurevitch, 2006). Invasive plant species can form dense thickets that shade out native plants. For example, the exotic shrub, *Lonicera maackii* (honeysuckle) can rapidly invade and overtake a site, forming a dense shrub layer that crowds and shades out the native herb *Impatiens capensis* (Miller and Gorchov, 2004). Other invasive plants inhibit native plants indirectly by releasing allelopathic compounds, such as monoterpenes or phenols (Ehrenfeld, 2006). For example, *Ailanthus altissima*, *Alliaria petiolata*, and *Microstegium viminium* strongly inhibited germination of native plants in the field (Nikki and Scott, 2010). My research focused on the impacts of an invasive annual grass, *Microstegium vimineum* (Trin.) A. Camus on a native annual herb, *Impatiens capensis* Meerb. I asked: 1) Can *Microstegium* invade *Impatiens* areas in the field? and, 2) Does the invasive grass out compete *Impatiens*, either directly or through indirect effects on soil nutrients (carbon, nitrogen).

COMPETITION BETWEEN INVASIVE AND NATIVE PLANTS

Invasive plants such as *Microstegium* frequently are reported to be superior competitors that show aggressive growth and greater allocation of carbon to aboveground tissues, relative to their native counterparts (Ehrenfeld, 2003). For example, Japanese knotweed, *Fallopia japonica* had greater aboveground biomass when it grew with native species than it grew by itself (Anna et al, 2010). In general, invasive plants can have direct impacts on native species through aboveground competition for resources, including space, light, and below ground resources such as, nutrients, water, and space (Woods, 1993; Schenk, 2006). Invasive plants that are successful competitors are likely to have major effects on native species in invaded communities (Yurkonis and Meiners, 2004). For example, exotic *Fallopia bohemica* (Bohemian knotweed) reduces growth or survival of native *Acer saccharinum* (silver maple) by reducing availability of light (Siemens and Blossey, 2007). Similarly, invasive plants such as *Alliaria petiolata* (garlic mustard) (McCarthy, 1997) and *Lonicera maackii* (honeysuckle) (Miller and Gorchov, 2004) decrease the survival, growth and reproduction of the native herb *Impatiens capensis*. *Lonicera maackii* produces dense shade and has been reported to have strong aboveground effects on forest flora (Miller and Gorchov, 2004). Below ground, invasive species can increase or decrease the concentration of resources available to roots of other species (Berger et al., 2008). It has been argued that, particularly in low productivity environments, belowground competition for nutrients is likely to be more important than aboveground competition for light in promoting the successful establishment and the persistence of invasive species (Dietz and Edwards, 2006). For example, Kueffer et al., (2007) showed that belowground competition suppressed the growth of native juvenile trees in forests dominated by the invasive tree *Cinammomum verum*. Growth rate of native *Liquidambar styraciflua* (sweet gum) was reduced by belowground competition from the invasive vine *Lonicera japonica* (Japanese honeysuckle) (Dillenburg et al., 1993). Further, the invasive shrub *Acacia cyclops* in South Africa lowered the growth of native shrubs *Rhus glauca (blue kuni-bush)* and *R. lucida* (waxy currant) (Midgley et al., 1992), and, in Australia, seedlings of the invasive *A. saligna* reduced growth rates of seedlings of the native shrub *Protea repens* (sugar bush) (Witkowski, 1991). Germination and growth of *Cirsium vinaceum*

(Sacramento Mountain thistle), a threatened endemic native plant of New Mexico, were reduced by the invasive *Dipsacus sylvestris* (teasel) (Huenneke and Thomson, 1995).

Besides competition for resources, indirect competition through alteration of the soil ecosystem can affect the establishment, spread, and persistence of invasive species in invaded communities (Margherita and Bruce, 2014). Vitousek (1990) pointed out that exotic species could alter the soil ecosystem. Numerous mechanisms have been identified by which plants can alter the physical, chemical, and biological properties of soils (Chapin et al., 1996). Many involve changes in the soil pH (Ehrenfield, 2001). For example, soils beneath two exotics *Berberis thunbergii*, a woody shrub, and *Microstegium vimineum*, had higher pH values than did soils beneath a native understory shrub, *Vaccinium pallidum* (Blue Ridge blueberry) *(*Ehrenfield, 2001). Higher ecosystem nitrogen pools caused by nitrogen fixation have been documented for the successful woody invaders *Morella faya* and *Falcataria moluccana* (Hughes and Uowolo, 2006). Both nitrogen and carbon mineralization rates in soil beneath an invasive plant (*Kochia scoparia*) were higher than beneath native graminoid species (Vinton and Burke, 1995). Similarly, *Melinis minuti flora,* an exotic grass invading Hawaiian shrub communities, stimulates higher rates of nitrogen mineralization than is observed under the native vegetation (Asner and Beatty, 1996). In contrast, Scott et al., (2001) found that nitrogen mineralization rates decreased beneath an invasive herb invading tussock grasslands in New Zealand.

IMPATIENS CAPENSIS AND *MICROSTEGIUM VIMINEUM* ECOLOGY

Impatiens capensis, commonly known as jewelweed is an annual herb (Wistendahl, 1958), that grows in ditches and along creeks and roadsides, often in monoculture stands. It grows in heterogeneous light environments (Schmitt, 1993). It produces both chasmogamous (outcrossing) and cleistogamous (self-fertilizing) flowers (Waller, 1984). Cleistogmous flowers are born on solitary pedicels in the lower and proximal leaf axils of all plants. Chasmogamous flowers are found on compound pedicels near the top of larger plants and at the tips of branches (Waller, 1980). *Impatiens* uses cleistogamy as its major reproductive mode: nearly 70% of the total seeds in a population are derived from cleistogamy (Lu, 2000). The energy cost of seeds produced by chasmogamous flowers in *Impatiens capensis* is roughly twice the cost of seeds produced from cleistogamous flowers (Waller, 1979). Both flower types produce seed pods (Schmitt et al., 1985) that explode with the slightest touch, giving the plant its other common name, touch-me-not (Dickinson et al., 2004). Seeds are produced from May to October depending, upon environmental conditions and overwintered seeds germinate during spring (Mary, 1979). Germination records for *Impatiens* (Barton, 1939) indicated the effective pretreatment for germination to be low temperatures for two to five months. *Impatiens* can reach height of 2 m (Schmitt et al., 1985). *Impatiens* was chosen for my research due to its availability, ability to measure reproduction in a single season, and common co-occurance with *Microstegium vimineum*. Previous research has shown that *Impatiens* species are negatively impacted by invasive plants (McCarthy, 1997; Gould and Gorchov, 2000). Invasive plant species such as *Alliaria petiolata* (garlic mustard) (McCarthy, 1997) and *Lonicera maackii* (honeysuckle) (Miller and Gorchov, 2004; Cipollini et al., 2008) have been shown to decrease the survival, growth and reproduction of, *I. capensis*.

Microstegium vimineum, also known as Japanese stiltgrass, bamboograss and Nepalese browntop, is an invasive, annual grass (Brown 1977) in the Poaceae family (Fairbrothers and Gray 1972). It is native to China, India, Japan, Korea, Malaysia, Nepal, Pakistan, Sri Lanka, and Taiwan (Williams, 1998; Tu, 2000). *Microstegium* is a highly invasive, shade tolerant C4 grass (Brown, 1977) that occurs in a wide range of habitats, including river banks, flood plains, woodland thickets, roadside ditches, forest edges, and deep forest understory sites in the eastern and mid western U.S. (Fairbrothers and Gray, 1972; Barden, 1987). *Microstegium* was introduced to the U.S. from southeast Asia in the early 1900's (Fairbrothers and Gray, 1972) and it now is listed as an invasive species in more than twenty states, mostly in the south eastern United States (Kleczewski et al., 2011). It was formerly used as packing material for imported Chinese porcelain, and discarded packaging material containing seeds might have been the source of the introduction (Fair brothers & Gray, 1972). *Microstegium* flowers in the fall (Derr 2004). It produces both cleistogamous flowers (closed and self-pollinating) and chasmogamous flowers (open and cross-pollinating), with all axillary racemes cleistogamous (Barden, 1987). Individual plants can produce 1000 seeds per year (Cheplick, 2010). The seeds germinates in spring (Flory et al., 2007) and may need stratification (cool temperatures with high moisture) to germinate (Woods, 1989). Ungerminated seeds can remain viable in the soil for as long as five years (Barden, 1991).

Abundant seed production, high survival, and fast growth rate of *Microstegium* all contribute to high densities that can impact the neighboring plants negatively (Flory et al., 2007). Although high densities of *Microstegium* can be found in shady or sunny environments, high light availability tends to enhance plant size and reproduction (Cole and Weltzin, 2005). *Microstegium* caused declines in density, height, biomass and reproduction of the native woodland herb, *Senna hebecarpa* (American *Senna*) (Jonathan and Luke, 2011). *Senna* was particularly susceptible to competition from *Microstegium* during the early establishment phase, and regenerating seedlings were inhibited by the dense layer of litter developed by senesced *Microstegium* (Ehrenfeld et al., 2001). A lower proportion of *Senna* plants produced fruit in invaded plots and plants that produced fruit had reduced seed production compared to non-invaded plots (Jonathan and Luke, 2011). Several studies have found that *Microstegium* caused declines of native plant species by altering soil properties (Ehrenfeld et al., 2001; McGrath and Binkley, 2009). *Microstegium* can change soil ecosystem processes, particularly nitrogen cycling and carbon cycling (Kourtev et al., 1998 and Ehrenfield et al., 2001). It was associated with declines in soil carbon pools across, eight bottomland forest sites (Strictland et al., 2010), and increased, net nitrification, and nitrogen mineralization compared to soils under a native understory shrub, *Vaccinium pallidum (*Ehrenfeldet al., 2001). *Microstegium* produces slow decomposing litter and can delay nitrogen release and alter competition with other native plants (Ehrenfeld et al., 2001)

MY RESEARCH

I conducted an observational field experiment in Kentucky and North Carolina where *Microstegium* and *Impatiens* were growing naturally. Interaction between *Impatiens* and *Microstegium*, was examined by comparing density (number of plants in each plot), height, number and biomass of fruit and stem biomass of *Impatiens* in invaded (*Microstegium* present) and uninvaded (no *Microstegium*) plots. Similarly, I compared density and biomass of *Microstegium* when growing by itself and growing with *Impatiens*. As concern about the negative effects of invasive species on native biodiversity is growing, my research provides new information for research-based conservation efforts. This information is especially useful for

Impatiens, a common forest herb that also is valued and grown for its ability to ward off poison ivy.

METHODS

SITE DESCRIPTION

My research was conducted in Kentucky and North Carolina where *Microstegium* and *Impatiens* were growing naturally. Four sites (37° 08' 21.64” N / 82° 44' 32.87” W) were chosen in Kentucky; were within a half mile of each other and near a road. One of sites was an old house site that had burned in the past and had soil rich in organic matter (personal observation). This site had a pure population of *Impatiens* (I), pure population of *Microstegium* (M) and mixed population of *Impatiens* and *Microstegium* (IM). A second site was also rich in organic matters (personal observation). This site had pure and mixed populations of *Impatiens* and *Microstegium*. The third site had a pure population of *Impatiens* with rich soil. The fourth site in Kentucky was located along a road in a forest. It had pure and mixed populations of *Microstegium* with sandy soil. All the sites in North Carolina were within a range of 3 miles from Western Carolina University. Three sites were located in Cullowhee (35° 18'N / 83° 10'W) and one site was located in Sylva (35° 22' N / 83° 13' W). One of the sites was beside a field with fertile soil and overhanging deciduous trees. All three combinations of *Impatiens* and *Microstegium* were present. A second site had sandy soil with a pure population of *Microstegium*. The third site in Cullowhee had a pure population of *Impatiens*. The fourth site in Sylva was along and within a stream beneath an approximately 70 year old mixed deciduous forest. It had a pure population of *Impatiens*. The soil was sandy.

PLANT SAMPLING METHODS

I established 25cm × 25cm plots within each site (180 plots total) in September, 2014, when plants had fruits and seeds were ready to disperse. Plots were established haphazardly for *Impatiens* growing alone (I), *Impatiens* growing together with *Microstegium* (IM) and *Microstegium* growing alone (M) (Table 1). The number of plants per plot varied from one to 63 (Table 1).

Table 1: Number of *Microstegium* and *Impatiens* plot types (I, M and IM) and density (number of plants per plot) in Kentucky (KY) and North Carolina (NC).

		Kentucky			**North Carolina**		
Species	**Plot types**	**Number of plots**	**Density**	**Mean density**	**Number of plots**	**Density**	**Mean density**
I	I	25	1 – 10	4	24	1 – 10	3
I	IM	22	1 – 10	5	21	1 – 10	2
M	M	22	7 – 63	41	21	33 – 63	47
M	IM	23	20 – 63	29	22	48 – 64	31

I = *Impatiens*, M = *Microstegium*, IM = *Impatiens* and *Microstegium*

Height and number of fruits for each individual *Impatiens* plants, and density (number of plants) were recorded in *Impatiens*-only (I) and mixed (IM) plots. *Microstegium* density (number of stems) was recorded in *Microstegium* only and mixed (IM) plots. Biomass was harvested in September, 2014, by clipping *Microstegium* and *Impatiens* near the soil surface in each plot. Plants were separated by species and counted to determine final density. Collected plants were placed in individual paper bags and returned to the lab. Plants were oven-dried at 60˚C for 48 hour, and then weighed to determine mass to the nearest one gram. *Impatiens* stems and leaves were weighed separately from fruits and seeds. *Microstegium* was weighed as, whole plants with fruits and seeds.

SOIL SAMPLING METHODS

Soil samples (4 cm deep and 100 cm^2 area) were collected from the plots where *Microstegium* and *Impatiens* were growing alone or together. One soil sample was collected from each site with all three plot types (I, IM and M) in Kentucky and North Carolina. There were 24 total samples; 12 from Kentucky and 12 from North Carolina. Collected soil was placed in labeled plastic bags. Samples were air dried for one week. The dried soil was ground in an electric grinder to provide uniform, fine soil texture, and sieved through 4 mm mesh. Two samples were prepared from each plot type from Kentucky and North Carolina. A total of 48 soil samples were prepared for analysis. Total soil carbon, nitrogen, and the C: N ratios were determined by combustion with an Elemental Vario EL III Elemental Analyzer. Sulfanilic acid samples (total 3 samples) were prepared as standards to calibrate carbon, and nitrogen levels in the sulfanilic acid.

DATA ANALYSIS

All statistical analyses were conducted using the computer programs Sigma Plot (version 11, Systat Software, Inc., San Jose, CA) and SPSS (SPSS, Inc., Chicago, IL). I analyzed each species separately. Regression was used to examine the relationship between *Impatiens* density and height, stem mass, fruit number, and fruit mass. Analysis of covariance (ANCOVA) with density as a covariate was used to test effects of *Impatiens* density (covariate), location (KY, NC), and plot types (I, IM) on the following response variables: *Impatiens* per plant height, per-plant stem mass, and per-plant fruit mass. Analysis of variance (ANOVA) was used to test effects of location (KY, NC), and plot types (I, IM) on per plant fruit number. I used ANOVA unless density had significant effect on dependent variable. All *Impatiens* data were log-transformed. Regression was used to examine the relationship between *Microstegium* density (number of plants per plot) and per plant mass. I used ANOVA to compare *Microstegium* per-plant mass between plot types (M, IM) and locations (KY, NC). All *Microstegium* data were log-transformed. I used ANOVA to examine differences in soil nutrient means (C, N, C: N) among plot types and between locations. Means are reported ± one standard deviation (SD).

RESULTS

MICROSTEGIUM

Mean *Microstegium* density (number of plants) was significantly lower in mixed plots, where *Microstegium* occurred with *Impatiens* compared to plots where *Microstegium* occurred alone (Fig. 1, Table 2). With Kentucky (KY) and North Carolina (NC) combined, *Microstegium* density averaged 31 ± 19.33 in plots with *Impatiens* and 55 ± 24.01 in monoculture plots. Mean *Microstegium* density did not differ between KY and NC, nor was there an interaction between location and plot types (Fig. 1, Table 2).

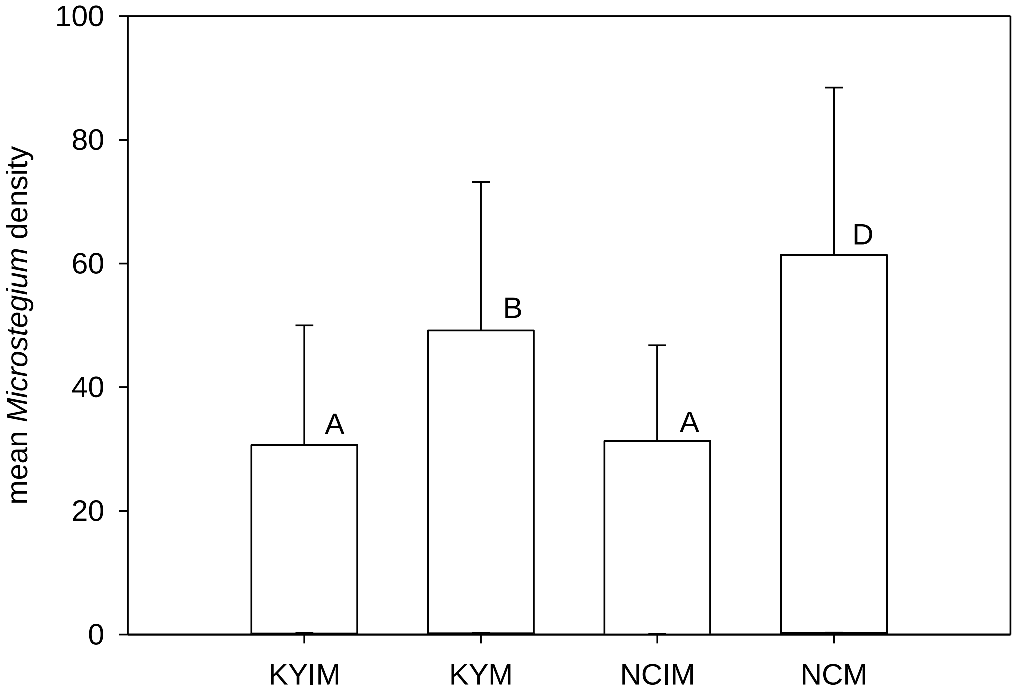


Figure1. Mean + one standard deviation of *Microstegium* density in monoculture (M) or mixed (IM) plots in Kentucky (KY) or North Carolina (NC). Means with the same letter do not differ significantly ($p > 0.05$).

Table 2: ANOVA of the effect of locations (KY, NC) and plot types (I, IM) on *Microstegium* density (number of plants in the plot).

Source	**d.f.**	**MS**	**F-value**	**P value**
location	1	0.305	0.97	0.327
plot type	1	9.5135	30.31	**<0.0001**
location*plot type	1	0.024	0.08	0.779

d.f. = degrees of freedom, MS = mean square

Linear regression of individual (per plant) mass against density (number of plants in a plot) revealed *Microstegium* mass is not density dependent when growing alone (r^2 = 0.0815, P = 0.141) or with *Impatiens* (r^2 = 0.00583, P = 0.627) (Fig. 2).

Significant differences in per plant *Microstegium* mass between plot types (M, IM) and locations (KY, NC) was revealed through ANOVA (Table 3). Mean *Microstegium* mass was significantly lower in mixed plots, where *Microstegium* occurred with *Impatiens* (Fig. 3, Table 3). In Kentucky, *Microstegium* mass averaged 0.15 ± 0.09g in plots with *Impatiens* and 0.17 ± 0.07g in monoculture plots. *Microstegium* mass averaged 0.07 ± 0.04g with *Impatiens* and 0.20 ± 0.10g without *Impatiens* in North Carolina. There was an interaction between location and plot types (Table 3). On average, Kentucky had greater *Microstegium* mass in mixed plots and North Carolina had greater *Microstegium* mass in *Microstegium* only plots.

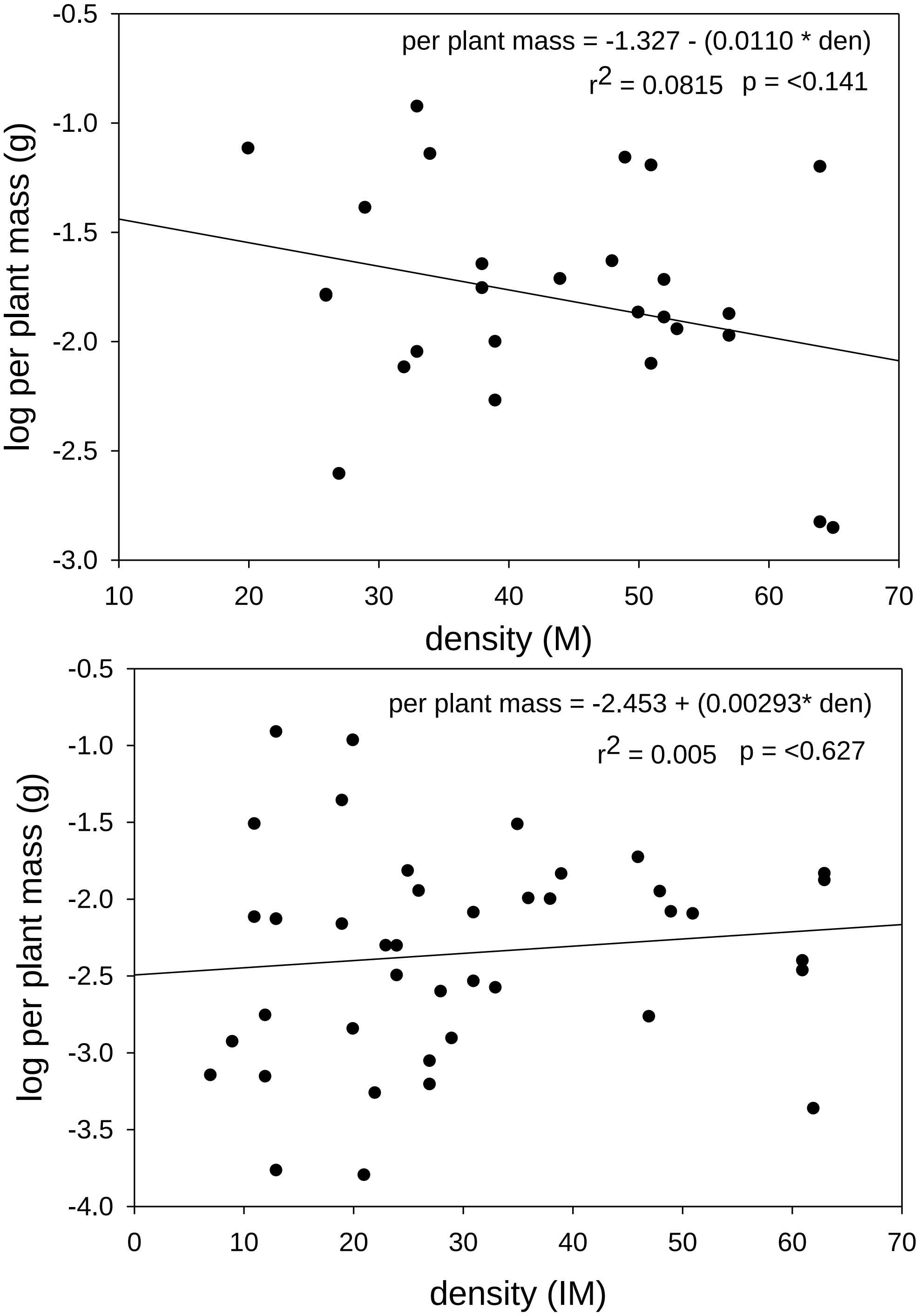


Figure 2. Regression of per plant mass of *Microstegium* against density when growing alone (M, top graph) or with *Impatiens* (IM, bottom graph).

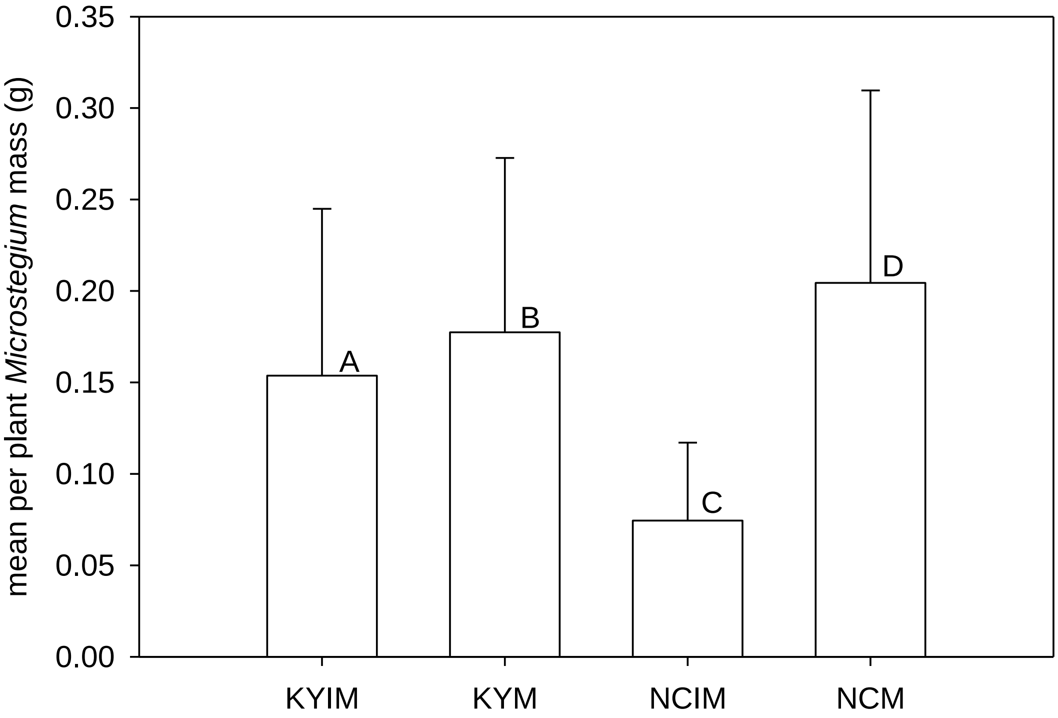


Figure 3. Mean ± one standard deviation of *Microstegium* mass (g) in monoculture (M) or mixed (IM) plots in Kentucky (KY) or North Carolina (NC). Means with the same letter do not differ significantly ($p > 0.05$).

Table 3: ANOVA of the effect of locations (KY, NC) and plot types (I, IM) on individual *Microstegium* mass (g).

Source	**d.f.**	**F**	**P**
location	1	4.65	**0.034**
plot type	1	24.06	**< 0.0001**
location*plot type	1	15.68	**0.0002**

d.f. = degrees of freedom, MS = mean square

IMPATIENS

Linear regression revealed per plant height of *Impatiens* is density dependent when growing alone (I) or with *Microstegium* (IM) (Fig. 4). Average height of individual *Impatiens* plants decreased highly ($r^2 = 0.619$) and significantly ($P < 0.001$) with density in *Impatiens* only plots (Fig. 4). Height of individual *Impatiens* decreased from an average of 3.8 ± 1.8cm in plots with only one *Impatiens* plant to an average of 1.8 ± 0.7cm with seven or more plants in *Impatiens* only plots (Fig. 4). In mixed plots, average height of *Impatiens* decreased weakly ($r^2 = 0.149$) and significantly ($P < 0.001$) (Fig. 4). Height of individual *Impatiens* decreased from an average of 4 ± 1.82cm in plots with only one *Impatiens* plant to an average of 2 ± 0.91cm in plots with eight or more plants in mixed plots (Fig. 4)

With density as a covariate, mean per plant height of *Impatiens* differed significantly among plot types (I, IM) (Fig. 5, Table. 4). Height of individual *Impatiens* plant averaged 24.80± 21.65cm in *Impatiens* only plots (I) and 33.41 ± 28.95cm in mixed plots (IM). Mean individual height differed significantly in Kentucky and North Carolina (Fig. 5, Table 4). Height of individual *Impatiens* plant averaged 20.84 ± 19.60cm in Kentucky and 43.11 ± 28.40cm in North Carolina (Fig. 5, Table 4). There was no significant interaction between plot types and locations (Table 4).

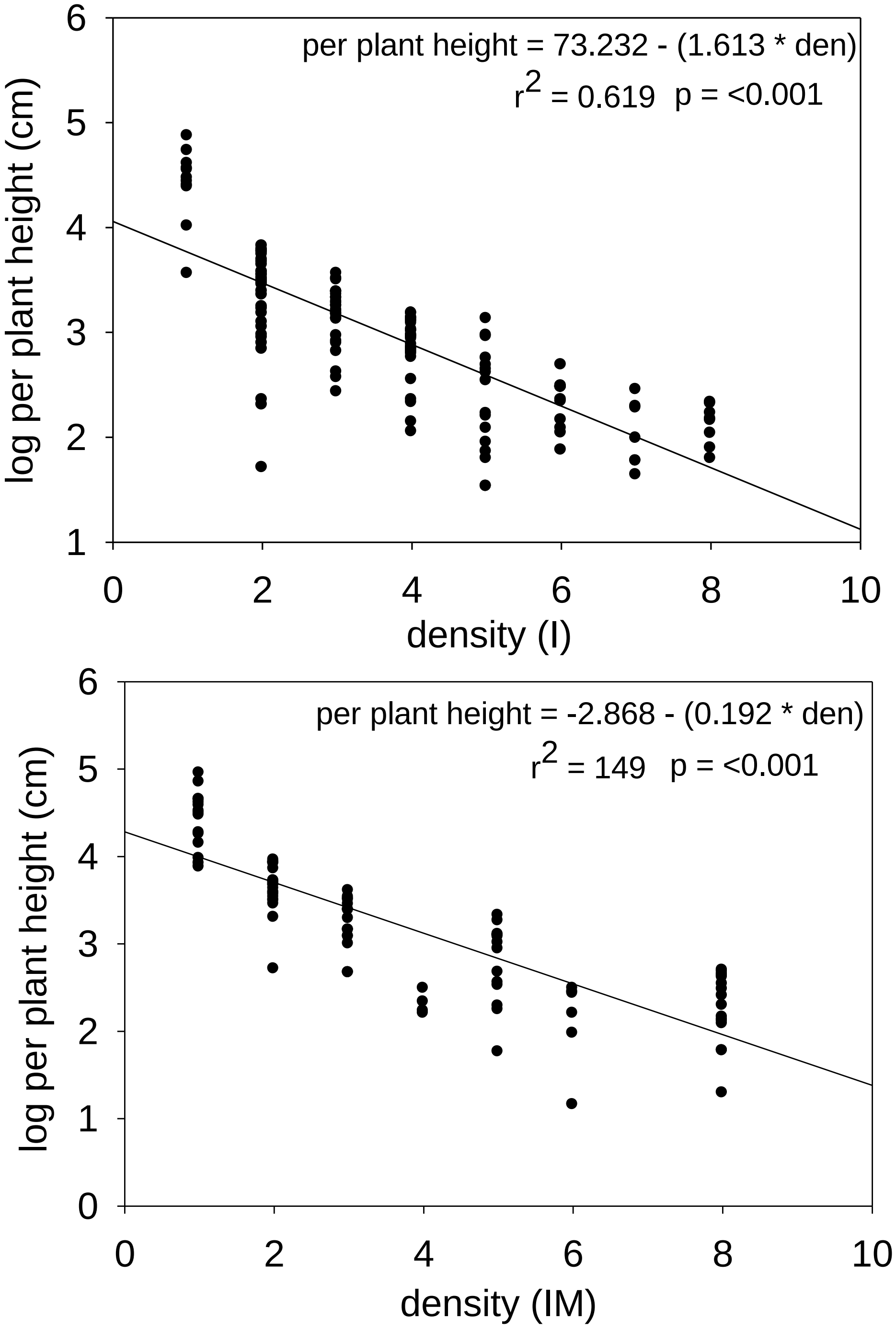


Figure 4. Regression of per plant height of *Impatiens* plant against density when growing alone (I, top graph) or growing with *Microstegium* (IM, bottom graph).

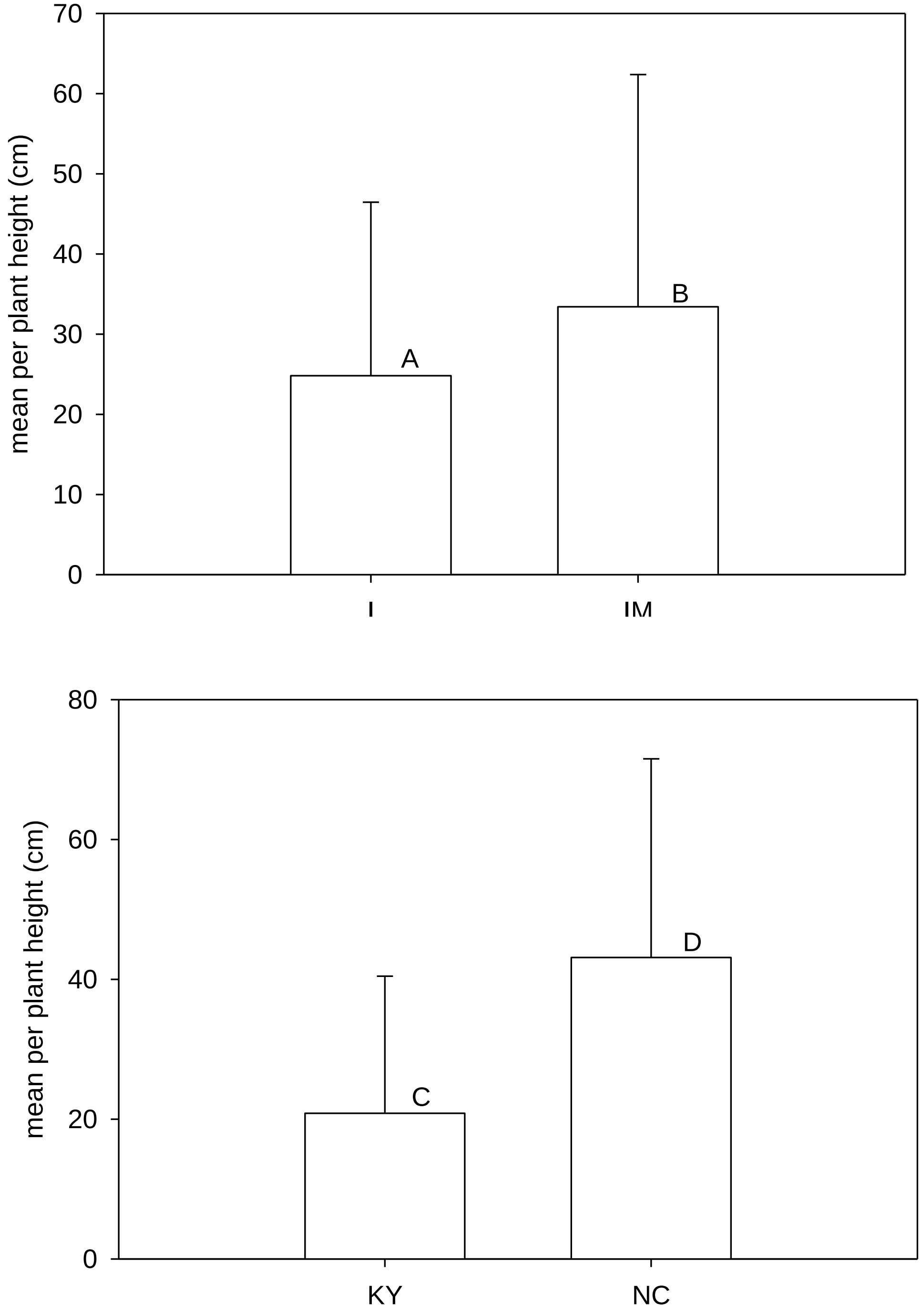


Figure 5: Mean ± one standard deviation of height (cm) of individual *Impatiens* plant when growing alone or growing with *Microstegium* (top graph) in Kentucky and North Carolina (bottom graph). Means with the same letter do not differ significantly ($p > 0.05$).

Table 4: ANCOVA of the effect of locations (KY, NC) and plot types (I, IM) on height of individual *Impatiens* plants.

Source	**d.f.**	**SS**	**F**	**P**
number of *Impatiens*	1	51.64	269.297	**<.0001**
location	1	2.179	11.362	**<.0001**
plot type	1	3.702	19.304	**<.0001**
location*plot type	1	0.086	0.447	0.504

d.f. = degrees of freedom, SS = sum square

Linear regression revealed stem mass produced by *Impatiens* was density-dependent when growing alone or with *Microstegium*. Average stem mass of individual *Impatiens* plants decreased weakly ($r^2 = 0.0757$) but significantly ($P < 0.001$) with density in *Impatiens* only plots (Fig. 6). Stem mass of individual *Impatiens* decreased from an average of 0.7± 0.18g in plots with only one *Impatiens* plant to an average of 0.3 ± 0.04g per plants with six or more plants in *Impatiens* only plots (Fig. 6). In mixed plots, average stem mass of *Impatiens* decreased more ($r^2 = 0.128$) and significantly ($P < 0.001$) (Fig. 6). Stem mass of individual *Impatiens* decreased from an average of 1 ±0.24g in plots with only one *Impatiens* plant to an average of 0.2 ± 0.05g per plants with eight or more plants in *Impatiens* and *Microstegium* plots (Fig. 6).

Mean per plant stem mass of *Impatiens* with density as a covariate differed significantly among plot types (I, IM) (Fig. 7, Table 5). Stem mass of individual *Impatiens* plants averaged 0.40 ± 0.09g in *Impatiens* only plots and 0.61 ± 0.78g in mixed plots (Fig. 7). Mean stem mass differed significantly between Kentucky and North Carolina (Fig. 7, Table 5). Stem mass produced by individual *Impatiens* plants averaged 0.53± 0.67g in Kentucky and 0.40 ± 0.79g in North Carolina (Fig. 7, Table 5). There was no significant interaction between plot types (I, IM) and locations (KY, NC) (Table 5).

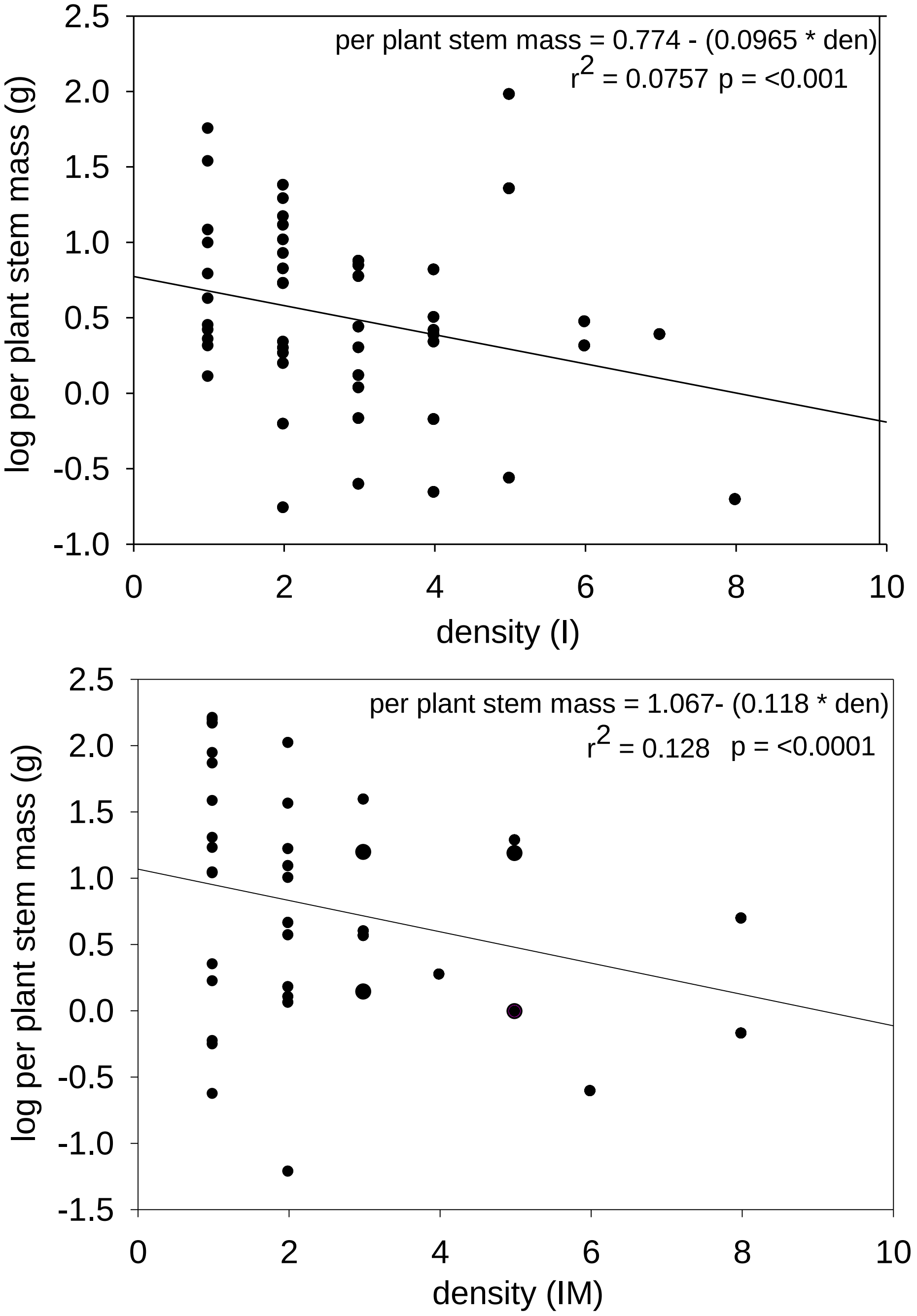


Figure 6. Regression of stem mass (g) produced by individual *Impatiens* plant against density when growing alone (I, top graph) or growing with *Microstegium* (IM, bottom graph).

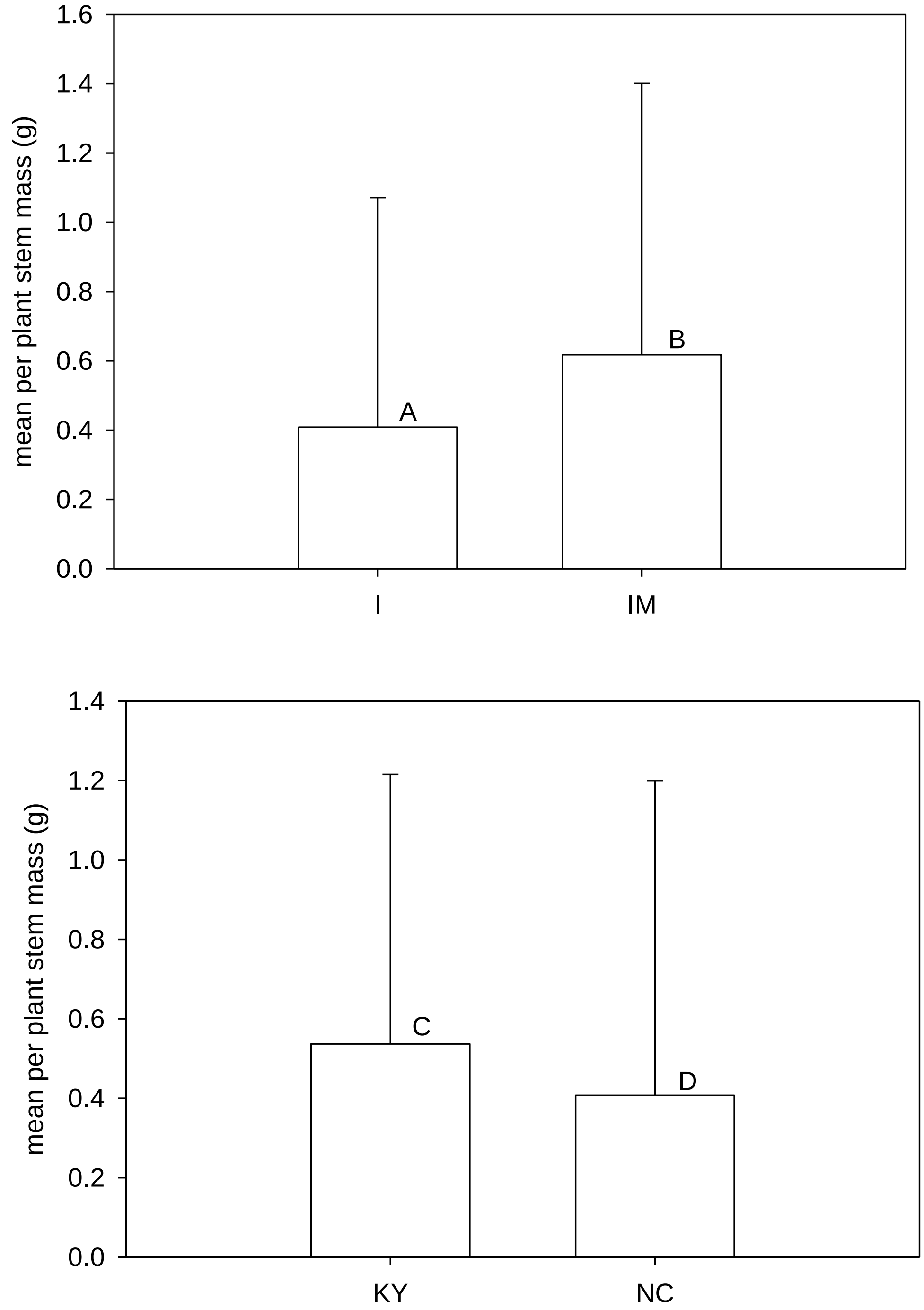


Figure 7. Mean ± one standard deviation of stem mass (g) produced by individual *Impatiens* plant when growing alone or growing with *Microstegium* (top graph) in Kentucky and North Carolina (bottom graph). Means with the same letter do not differ significantly ($p > 0.05$).

Table 5: ANCOVA of the effect of locations (KY, NC) and plot types (I, IM) on stem mass (g) of individual *Impatiens* plants.

Source	**d.f.**	**SS**	**F**	**P**
number of *Impatiens*	1	18.479	44.158	**<.0001**
location	1	6.342	15.156	**<.0001**
plot type	1	2.633	6.292	**<.0001**
location*plot type	1	1.433	3.425	0.65

d.f. = degrees of freedom, SS = sum square

Linear regression revealed number of fruit produced by *Impatiens* is density dependent when growing alone (Fig. 8). Average number of fruits produced by individual *Impatiens* plants decreased weakly ($r^2 = 0.0715$) but significantly ($P = 0.002$) with density in *Impatiens* only plots (Fig. 8). Fruit production decreased from an average of 2.7 ± 0.9 fruits in plots with only one *Impatiens* plant to an average of 2.1 ± 0.5 fruit per plants with six or more plants. In mixed plots, mean number of fruits produced per plants was not related to density ($r^2= 0.003$, $P = 0.57$) (Fig. 8).

Average number of fruits produced by *Impatiens* did not differ between plot types and locations (Fig. 9, Table 6) and there was no interaction between locations and plot types. Average fruits produced by *Impatiens* differed significantly between locations with 2.23 ±1.14 in Kentucky (KY) and 1.77 ± 1.08 in North Carolina (NC) (Fig. 9, Table 6).

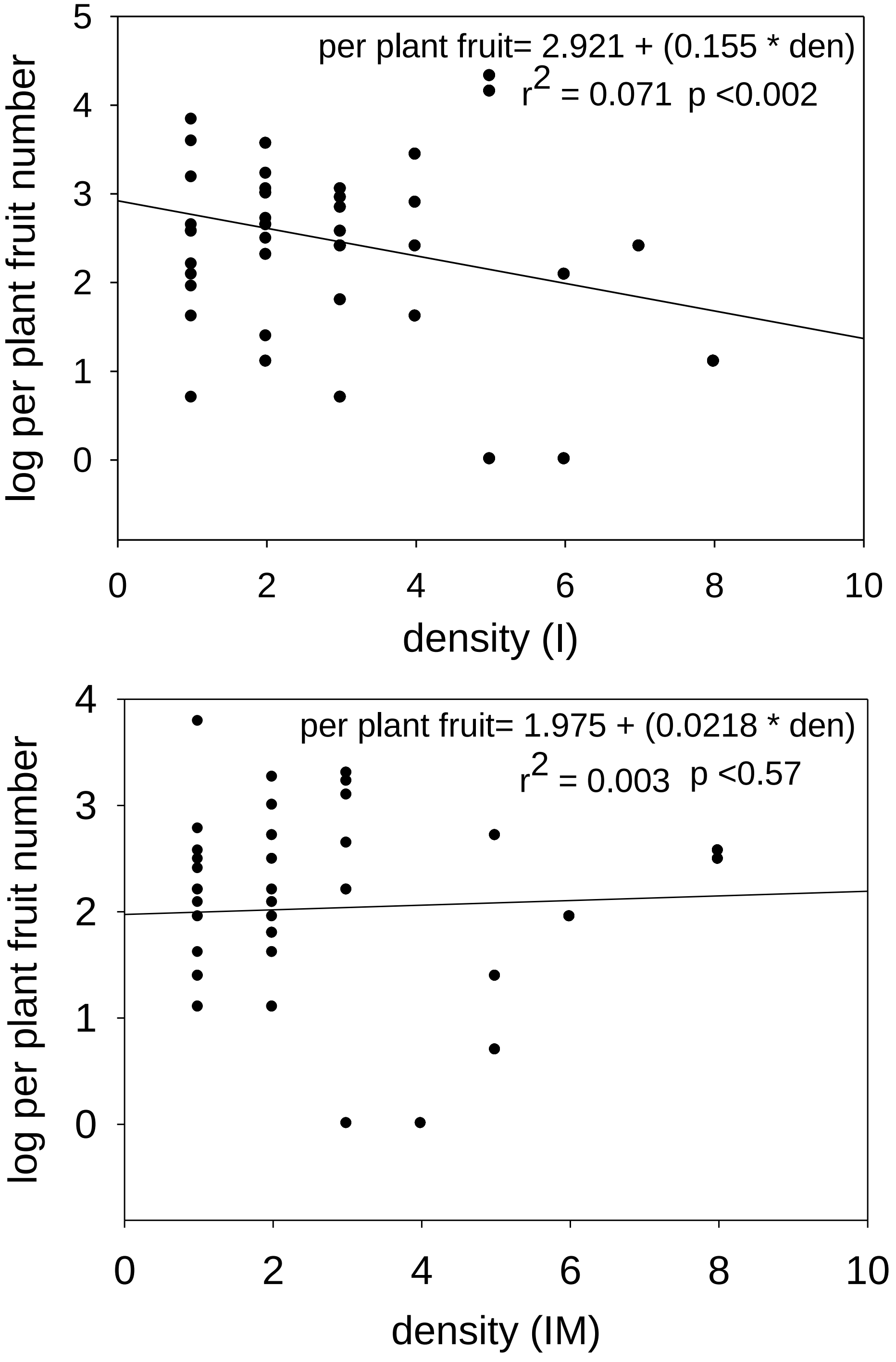


Figure 8. Regression of number of fruits produced by individual *Impatiens* plants against density when growing alone (I, top graph) or growing with *Microstegium* (IM, bottom graph).

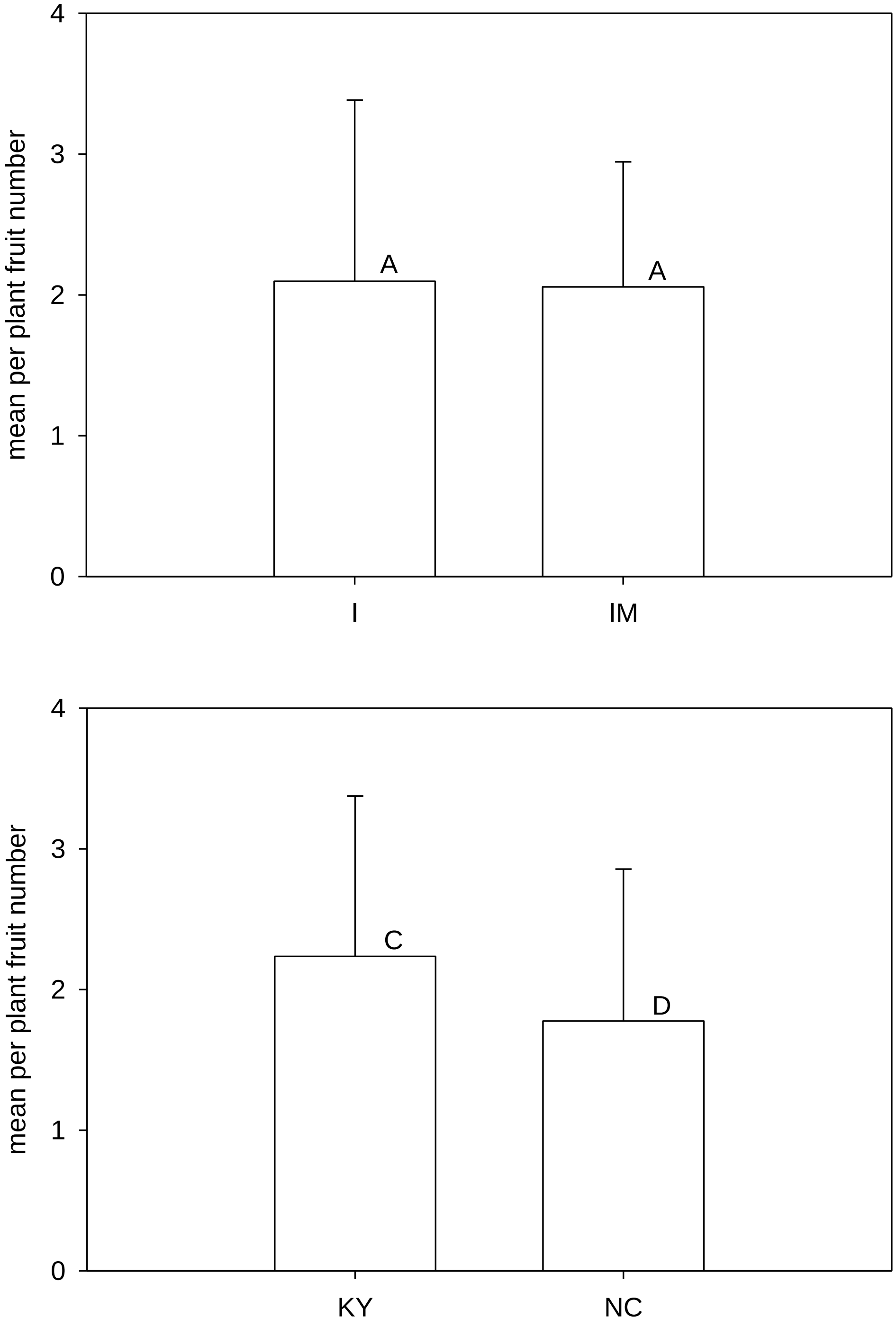


Figure 9. Mean ± one standard deviation of fruits produced by *Impatiens* when growing alone or growing with *Microstegium* (top graph) in Kentucky and North Carolina (bottom graph). Means with the same letter do not differ significantly ($p > 0.05$).

Table 6: ANOVA of the effect of locations (KY, NC) and plot types (I, IM) on number of fruits produced by individual *Impatiens* plants.

Source	**d.f.**	**SS**	**F**	**P**
location	1	6.656	5.389	0.021
plot type	1	3.506	0.14	0.709
location*plot type	1	7.238	5.86	0.16

d.f. = degrees of freedom, SS = sum square

Linear regression revealed log per plant fruit mass produced by individual *Impatiens* is density dependent when growing alone. Average fruit mass of individual *Impatiens* plant decreased (r2 = 0.143) significantly ($P < 0.001$) with density in *Impatiens* only plots (Fig. 10). Log fruit mass of individual *Impatiens* decreased from an average of -2.2 ±0.25g in plots with only one *Impatiens* plant to an average of -5 ± 0.02g per plants with eight or more plants in *Impatiens* only plots (Fig. 10). In mixed plots, mean fruit mass produced per plants was not related to density (r^2= 0.007, P = 0.336) (Fig. 10).

With density as a covariate, average fruit mass (g) of individual *Impatiens* did not differ between plots with *Impatiens* only and those with *Impatiens* and *Microstegium* (Fig. 11, Table 7). Average fruits mass differed significantly between locations with3.6 ±1.70g in Kentucky (KY) and 2.8 ± 1.73g in North Carolina (NC) (Fig. 11).

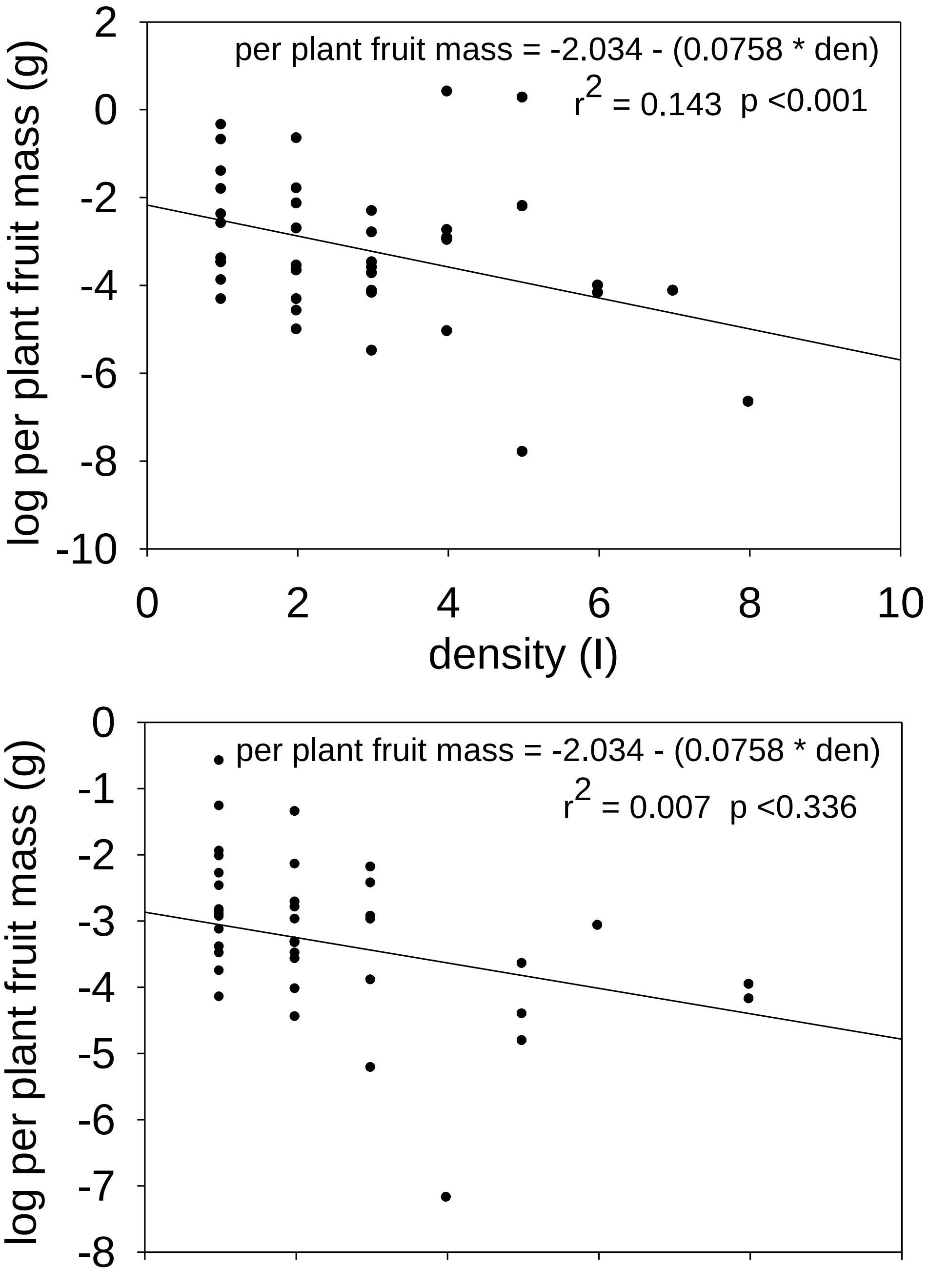
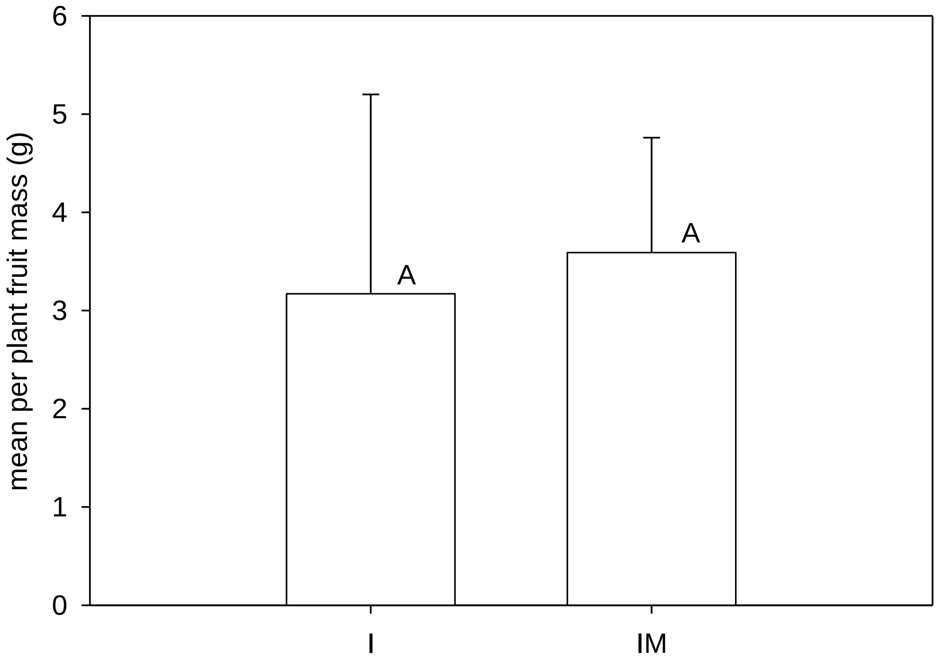


Figure 10. Regression of fruits mass (g) produced by individual *Impatiens* plant against density when growing alone (I, top graph) or growing with *Microstegium* (IM, bottom graph).



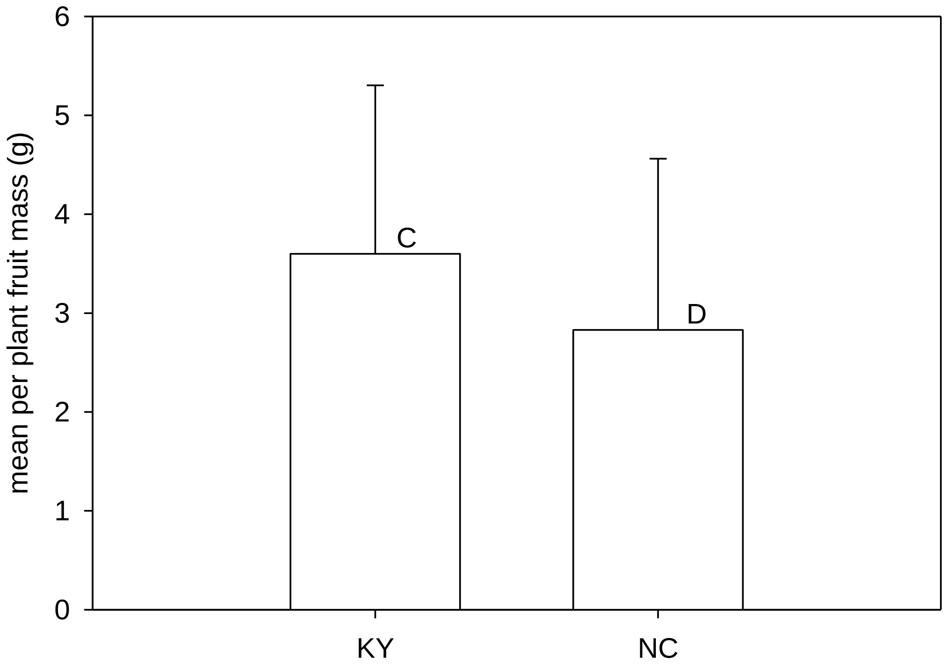


Figure 11. Mean ± one standard deviation of fruit mass (g) produced by individual *Impatiens* when growing alone or growing with *Microstegium* (top graph) in Kentucky and North Carolina (bottom graph). Means with the same letter do not differ significantly ($p > 0.05$).

Table 7: ANCOVA of the effect of location (KY, NC) and plot type (I, IM) on fruits mass (g) of individual Impatiens plants.

Source	**d.f.**	**SS**	**F**	**P**
number of *Impatiens*	1	57.133	21.145	**<.0001**
location	1	0.197	0.073	0.001
plot type	1	9.523	3.524	0.062
location*plot type	1	0.386	0.143	0.706

d.f. = degrees of freedom, SS = sum square

SOIL

There was no significant difference in soil nitrogen (% N), carbon (% C), and C: N ratios between monoculture plots (I, M) and mixed plots (IM) (Fig. 12, Table 8). Soil carbon and C: N, ratios differed between KY and NC (Fig. 12, Table 8). Soil carbon averaged 6.1 ± 0.94% in (KY) and 4.3 ± 0.14% in (NC) (Fig. 12). Mean C: N ratios averaged 17.5 ± 1.62% in KY and 12.9 ± 0.52% in NC (Fig. 12). Mean soil nitrogen did not differ between KY and NC (Fig. 12).

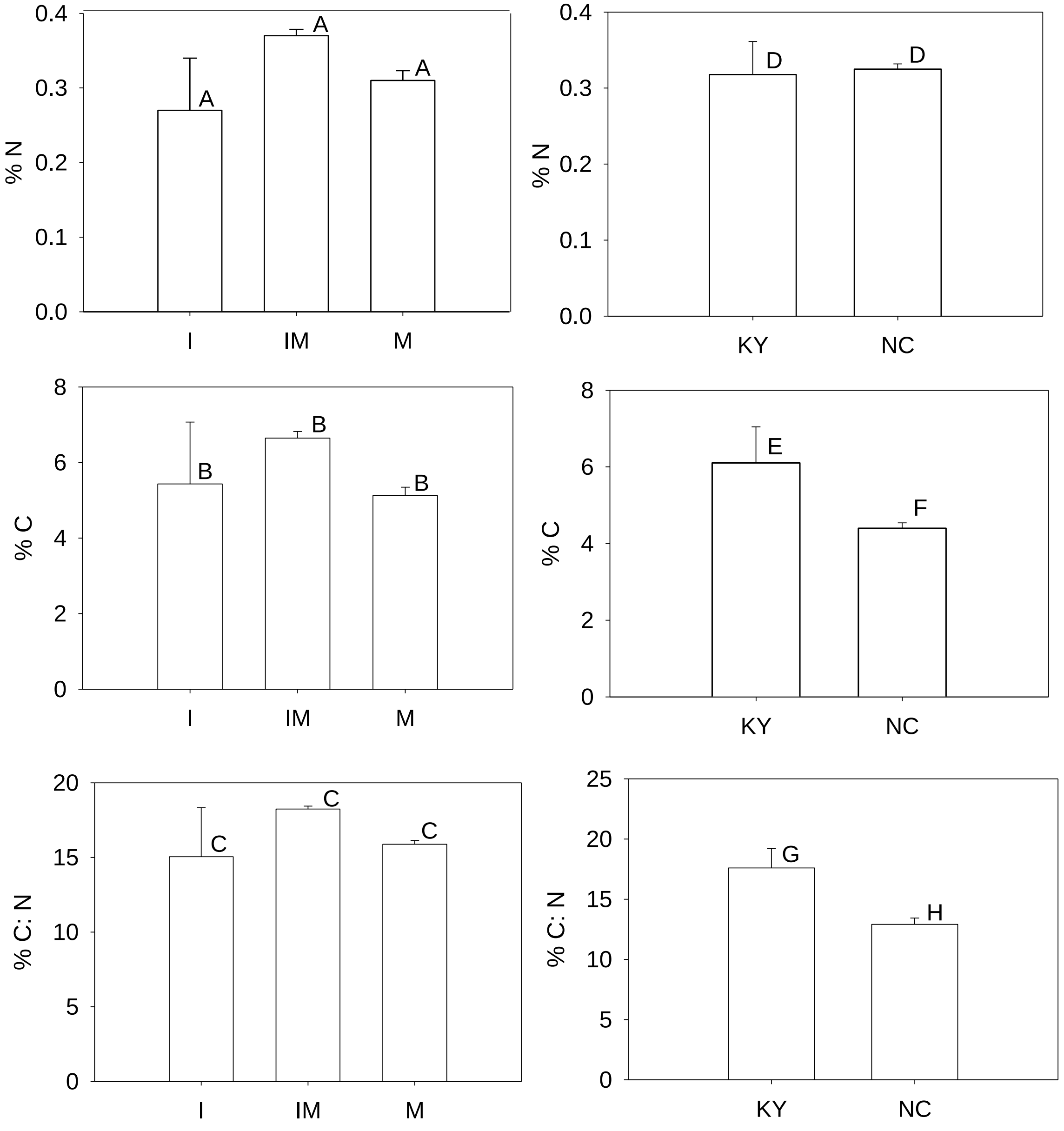


Figure 12. Mean + one standard deviation of soil carbon, nitrogen and C: N ratios in monoculture (I, M) or mixed (IM) plots in Kentucky (KY) or North Carolina (NC). Means with the same letter do not differ significantly ($p > 0.05$).

Table 8: ANOVA of the effect of location (KY, NC) and plot types (I, M, IM) on total soil nitrogen, carbon and C: N ratios.

	% N			**% C**			**C:N**		
Source	**d.f.**	**F**	**P**	**d.f.**	**F**	**P**	**d.f.**	**F**	**P**
loc	6	13.69	**<.0001**	6	14.92	**<.0001**	6	6.06	**0.003**
plot type	2	1.63	0.234	2	1.06	0.375	2	2.67	0.106
loc*plot type	4	8.31	**0.001**	4	3.91	**0.026**	4	1.03	0.427

loc = location, N = nitrogen , C = carbon, C:N = carbon, nitrogen ratio

DISCUSSION

In contrast to my hypothesis that the invasive species would be the stronger competitor, the native *Impatiens* suppressed growth and survival of the invasive grass *Microtegium*; both mass and density of *Microstegium* were lower in plots where they grew with *Impatiens* than in plots where they grew alone. In addition, *Impatiens* plants were taller and had higher stem mass in mixed plots with *Microstegium*. Further, number of fruits and fruit mass of *Impatiens* did not differ significantly when plants were growing with *Microstegium*. These results contrast with previous observational (Oswalt et al., 2007; Adams and Engelhardt, 2009) and experimental studies (Flory and Clay, 2009; Flory and Clay, 2010), which found that *Microstegium* presence causes reductions in biomass of native species.

Lower density and mass of *Microstegium* in mixed plots suggests, the taller *Impatiens* reduced light penetration and out shaded *Microstegium*. When there is competition for light between a plant and its neighbors, height growth will help individual plants avoid neighbor shade and enable it to acquire more of the light resource (Schmitt and Wulff, 1993). Ehrenfeld et al., (2001) found that reduced light availability suppressed *Microstegium* seedling growth and survival. Patrice and Jake (2005) also reported that light reduction by the native tree *Asminia triloba* prevented establishment of *Microstegium* beneath its canopy. Although, *Microstegium* is a shade-tolerant (Horton and Neufield, 1998), invasive species, its germination, seedling establishment, growth, and reproduction are positively correlated with canopy openness and light penetration, and the species occurs less frequently, and grows poorly, in shade (Barden 1996; Cole and Weltzin, 2004). Further, germination, emergence, and initial root and shoot development may be particularly sensitive to competition (Foster, 1999). It has been shown that small seedlings can be much more sensitive to competition than adult plants (Foster, 1999; Suding and Goldberg, 1999). Other research, however, has indicated that competition may be relatively rare early after germination, but may be critical in determining the final plant mass (Goldberg et al., 2001). This highlights the need to investigate when during its establishment and growth, *Microstegium* is most sensitive to shade and competition from *Impatiens*. Differences in phenology, specifically, earlier emergence and faster height growth of *Impatiens*, may contribute to early negative effects on *Microstegium*. This suggestion is supported by a lack of density-dependence in *Microstegium.* Intraspecific competition for resources has been reported among *Microstegium* plants (Flory and Clay, 2010); the lack of this response in my research suggests plants that survive beyond initial self-thinning may not show further decreased growth when growing at roughly the same rate as other individuals in a single species plot. However, earlier germination or faster growth of *Impatiens* may cause initial *Microstegium* mortality and reduce subsequent plant growth.

Decreasing *Impatiens* height and stem mass with increasing density in both single-species and mixed plots indicates density-dependence with respect to light or soil resources. That is, like *Microstegium*, *Impatiens* growth and reproduction were negatively affected by greater shade or root-crowding. Only fruit number and mass did not show density dependence when growing with *Microstegium*. Weiner and Freckleton (2010) also found that the vegetative growth of *Impatiens* decreases with density. Stem elongation in response to crowding, using simulated foliage shade, has been shown in *Impatiens* (Dudley and Schmitt, 1996) and its close relatives *I. pallida* (Weiner et al., 1990) and *I. Parviflora* (Young, 1981). In the absence of competition, stem elongation can be maladaptive because of the cost of allocating carbon to stems and decreased mechanical strength of the elongated stem (Casal et al., 1994). *Impatiens* would not be expected to elongate when it is receiving optimum light (Schmitt and Wulff, 1993)

In support of this expectation, shorter *Impatiens* in Kentucky compared to North Carolina might be due to higher canopy openness in the Kentucky plots (personal observation).

In contrast to what may have been expected if *Impatiens* growth were reduced by competition from *Microstegium*, *Impatiens* plants had greater mass and were taller in plots with *Microstegium* than in the plots with *Impatiens* alone. In general, invasive species have been shown to reduce the mass of native plants (reviewed by Vila and Weiner, 2004). Early *Impatiens* emergence or initial growth could simply put it above *Microstegium*. Also the two species have very different architecture. There has been increasing realization that competition can be related to differences in plant architecture (Bazzaz and Tremmel, 1993). Architectural variation found between *Microstegium* and *Impatiens* can influence competition through their effects on interception of light (Bendix et al., 2010). Morphological characteristics such as leaf inclination, shape, size, and arrangement affect competition for light (Clements et al., 1992). Plants with horizontal leaves and larger surface area have a greater shading effect on neighboring plants (Loomis et al., 1971). Here, *Impatiens* has more horizontal and broad leaves, which cast shade on competitors such as *Microstegium*, but its more open growth form could reduce self-shading. In contrast *Microstegium* has more vertical or running growth form with more upright leaves that intercept light inefficiently when growing with *Impatiens* (Bazzaz and Tremmel, 1993)

Greater mass of *Impatiens* in plots with *Microstegium* might have been due, in part, to indirect effects of *Microstegium* on soil characteristics. Flory and Jonathan (2014) found that *Microstegium* created soil conditions favorable for another nonnative invasive, *Alliaria petiolata* (garlic mustard), when the species were grown together. Invaded habitats can be nitrogen limited, and there is interest in whether the persistence of *Microstegium* can be attributed to its alteration of soil nitrogen content (Marissa et al., 2012). I found soil nitrogen was not altered by *Microstegium*. Neither soil nitrogen nor carbon differed between single species and mixed species plots. This contrast with previous research (Ehrenfeld et al., 2001), which found greater soil nitrogen beneath *Microstegium* than beneath native species. Soil nitrogen did not differ between Kentucky and North Carolina. However, Soil carbon content of Kentucky was higher than North Carolina. Soil carbon positively influenced the growth of *Impatiens* (Shane et al., 2002). Previous research has shown that *Microstegium* litter has a higher C: N ratio, decomposes slower, and immobilizes more nitrogen than litter from uninvaded forests (Ehrenfeld et al., 2001). My research, in contrast, showed that neither *Microstegium* nor *Impatiens* affected C: N ratios of the soils in which they grew. However, there was a difference in soil C: N between Kentucky and North Carolina, with higher C: N in Kentucky soil suggesting faster rates of soil carbon production (Liam et al., 2005).

CONCLUSIONS AND FUTURE RESEARCH

In contrast to previous research that found substantial negative effects of *Microstegium* on native plant communities (Flory et al., 2007; Jonathan and Luke, 2011; Ehrenfeld et al., 2001), this research has provided important (and rare) evidence that there are no negative impacts of this invasive grass on the native herb *Impatiens capensis* over a growing season. Both density and mass of *Microstegium* were less in plots with *Impatiens* which suggests this native herb suppressed the growth and survival of *Microstegium*. *Impatiens* grew taller than *Microstegium* in the mixed plots and at harvest had greater stem mass compared to plants in the *Impatiens* only plots. Further, *Microstegium* had lower density and stem mass in mixed plots. Competition between *Microstegium* and *Impatiens* might be affected by their architecture. With its more horizontal leaves, *Impatiens* shade *Microstegium* plants beneath its canopy. Shorter *Microstegium* with more upright leaves had no pronounced effect on taller *Impatiens* with horizontal leaves. I did not find evidence that competition between the species was due to interactions of the plants with soil. Further research is needed to determine if there are longer term effects of *Microstegium* on the soil ecosystem. It would be interesting to compare the litter decomposition rates between these species.

Overall, the higher competitive ability of the native herb and decrease in density of the invasive grass suggests that, in this case, native biodiversity is not decreased by an invasive grass. Further, the constant fruit number and mass of *Impatiens* when growing alone or growing with *Microstegium* suggests that there is no risk of extinction of *Impatiens*. In the future, germination and early growth timing in *Microstegium* and *Impatiens* should be monitored to determine early effects of these species on the other. Also, investigating the architectural features such as branching pattern, leaf surface area and ecophysiology of these species would be helpful to understand their interaction.

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