

**RESPONSE OF KUDZU (*PUERARIA MONTANA* VAR. *LOBATA*)  
TO DIFFERENT TYPES AND LEVELS OF SIMULATED  
INSECT HERBIVORE DAMAGE**

by

Matthew J. Frye

A dissertation submitted to the Faculty of the University of Delaware in  
partial fulfillment of the requirements for the degree of Doctor of Philosophy in  
Entomology and Wildlife Ecology

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## ABSTRACT

Kudzu, *Pueraria montana* var. *lobata* (Fabaceae), is an invasive alien vine and the target of a classical biological control program in the United States. Simulated herbivory experiments were undertaken to examine how kudzu might respond to biomass removal by potential biological control agents, including defoliators, shoot-clippers, stem and root borers, near the center (North Carolina) and edge (Delaware/Pennsylvania) of the plant's invasive range.

A two-year study in established kudzu patches in Delaware and Pennsylvania found no differences in above-ground biomass for plants subject to 50 or 75% leaf or shoot damage, drilling one or two 10.24 cm deep holes from the rootcrown into roots, and a no-damage control. Survival to year two was 86 and 50% for plants in Delaware and Pennsylvania, and above-ground biomass at both sites decreased after the first year. Plants that did not survive had significantly smaller rootcrowns than plants that survived to year two. In North Carolina, completely removing 50% of vines per plant resulted in significant reductions in above-ground biomass over a three year period, but no differences were found for 50% leaf cutting and drilling one 10.24 cm hole from the rootcrown into roots compared to the control. Plant survival in North Carolina was 100%, and above-ground biomass of plants increased each year. A greenhouse experiment compared plant response to herbivory when grown in 0, 60, and 100% direct light to test the effect of light on plant survival. Emergence of plants was equal across light environments, but plant survival in 0%

direct light was only 2%. In 60 and 100% direct light, plants compensated for 50% leaf damage, but 75% leaf damage reduced above-ground biomass at both light levels.

To standardize the growing environment, the effects of 50 and 75% leaf and shoot damage were investigated in an agricultural field with transplanted kudzu plants over a two-year period. Above- and below-ground biomass, total vine length and average internode length of plants grown for one season in Delaware were significantly reduced by 75% leaf cutting. In North Carolina, 75% leaf cutting significantly reduced above- and below-ground biomass compared to the control for both one and two year old plants.

Plant architecture and biomass were investigated for plants subject to either 75% leaf cutting, actual herbivory imposed by the silver-spotted skipper, *Epargyreus clarus* (Hesperiidae), or a no-damage control in an agricultural setting. High levels of simulated leaf damage altered plant architecture compared to the control and actual herbivory treatment by producing a lower percentage of primary vines, shorter secondary branches, and shorter internodes on secondary and tertiary branches, as well as reductions in above- and below-ground biomass.

Taken together, the results of these studies indicate that high levels of leaf damage and 50% complete vine removal are capable of suppressing kudzu growth. A relationship between plant productivity, resource allocation and survival in established kudzu patches has been identified, and suggests that control efficacy will be enhanced in shaded habitats where plants accumulate fewer resources. This work suggests that evaluation of potential biological control agents should focus on host specific defoliators and stem borers, and that an integrated approach to shade existing patches should be considered.

## Chapter 1

# PLANT PRODUCTIVITY AND THE INFLUENCE OF LIGHT AVAILABILITY: IMPLICATIONS FOR BIOLOGICAL CONTROL OF KUDZU

## INTRODUCTION

Invasive, exotic species threaten the health and proper functioning of natural ecosystems worldwide (Mack et al., 2000), and are second only to habitat destruction for losses in biodiversity (Walker and Steffen, 1997). One species that has achieved great notoriety as an exotic invader is kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & S. M. Almeida ex Sanjappa & Predeep (Suddath) (Fabaceae), which was intentionally introduced to the United States in 1876 at the Centennial Exposition in Philadelphia (Mitich, 2000) and was planted extensively for erosion control in the 1930s and 1940s (Miller and Edwards, 1983). Today, kudzu infests an estimated two to seven million acres (Britton et al., 2002), and the range of this weed is predicted to increase with global climate change (Jarnevich and Stohlgren, 2009).

Kudzu is a fast growing vine that competes with native plants for light resources (Everest et al., 1991). As early as the 1950s it was recognized as a weed, yet large-scale control of the plant remains a problem. Mechanical removal using machinery is difficult, since kudzu was intentionally planted on hillsides and slopes for soil stabilization (Everest et al., 1991). Grazing by goats has been more successful in

these settings (Bonsi et al., 1991), but efficient use of this technique requires penning and relocating animals when foliage is overgrazed (Everest et al., 1991; Mitich, 2000). Furthermore, woody vines that penetrate the canopy of trees are not fed upon, and these vines can persist, grow, and continue to damage native plant species (Blackwell, 1973). Although time and labor intensive, removing individual plants by cutting below the rootcrown can provide complete control (Kudzu Coalition, 2010; personal observation).

For large-scale infestations, kudzu control typically relies on herbicide applications. To date, a number of herbicides are available and selection of an appropriate chemistry depends on characteristics of the infested site (Everest et al., 1991; Harrington et al., 2003). Also true for grazing, repeated treatment with herbicides over several years is needed, presumably to deplete stored carbohydrates in tuberous roots (Everest et al., 1991; Miller and Edwards, 1983). Integrated control using several methods, such as application of herbicides to mechanically cut rootcrowns (Thomas, 2000), or combining burning with reforestation (Harrington et al., 2003) have also been shown to enhance suppression provided by herbicides. Recent control efforts have focused on the use of the fungal pathogen *Myrothecium verrucaria* (Alb. & Schwein) Ditmar:Fr. as a bioherbicide to kill kudzu. In naturally occurring, mature stands this bioherbicide has been shown to rapidly kill kudzu foliage (Boyette et al., 2008). However, few reports have discussed the impact of this foliar spray on kudzu roots and how application affects plants after one year. Furthermore, only recently has a formulation been generated that does not appear to produce large quantities of mycotoxins (Boyette et al., 2008), whereas previous work highlighted risk to humans and other mammals from these products (Anderson and Hallett, 2004).

An alternative management technique that has been used for invasive species is biological control, which relies on natural enemy populations to reduce damage caused by pest species (Van Driesche et al., 2008). Biological control acts directly on the target weed, and is therefore more specific than large scale herbicide applications or mowing. However, time and funding may not be available to develop this science for a particular weed problem, and there is no guarantee that introduced agents will reduce plant growth or reproduction (e.g., Myers and Risley, 2000). Thus, in recent years simulated herbivory studies have been used to determine *a priori* the type, amount, and frequency of damage necessary to suppress pest plant densities in the introduced range of a weed (Raghu and Dhileepan, 2005).

Previous experiments have shown that plant response to simulated herbivory can differ based on the availability of resources such as light (Milbrath, 2008; Pierson et al., 1990), nutrients, and water content (Sun et al., 2009). Results from studies that manipulate resource levels can be used to better understand observations from the natural world. For example, in China it was noted that the leaf beetle *Agasicles hygrophila* Selmán and Vogt (Chrysomelidae) introduced for biological control of alligator weed, *Alternanthera philoxeroides* (Martius) Grisebach (Amaranthaceae) successfully reduced weed stands in aquatic, but not terrestrial habitats (Sun et al., 2009, and references therein). Sun et al. (2009) conducted a fully factorial experiment manipulating nutrient, soil moisture levels, and percent defoliation of plants to show that the observed difference in control efficacy was due to the ability of terrestrial plants to compensate for leaf tissue removal. Similar experiments that highlight differential response of plants to herbivory are an important, but often lacking component of prerelease efficacy studies in biological control.

In the present study, we investigated the growth response of individual kudzu plants to different types of simulated herbivore damage as part of a biological control program targeting this weed in the United States (Britton et al., 2002). This study was conducted over several seasons in established kudzu patches near the center (North Carolina) and edge (Delaware and Pennsylvania) of kudzu's invasive range, and investigated plant response to leaf, root and shoot damage. Field experiments were supplemented with a greenhouse study to examine emergence, survival, and the growth response of seedlings to different light exposures in combination with leaf cutting treatments. A companion study reported elsewhere (Chapter 2), examined the response of newly established kudzu plants to two levels of leaf and shoot damage in an agricultural field, where plants experienced full light conditions and no competition from herbivores or plants. The combined results of these studies will contribute to prioritizing potential biological control agents in China based on their feeding guild or damage type.

## **MATERIALS AND METHODS**

### **Delaware and Pennsylvania Field Experiment**

In 2007, a one-year pilot study was conducted in an established kudzu patch in Dover, DE (39° 10' 56.97" N, 75° 32' 46.76" W). The site is located alongside the Fork Branch of the Saint Jones River, with half of the kudzu patch on flat, level ground and the other half on the stream embankment. A thin layer of loamy, organic material is present above sandy soil, which is characteristic of the coastal plain in DE (McAvoy and Bennett, 2001). The study area at the site was relatively open, except for

a large black walnut tree (*Juglans nigra* L.) at the southwest edge of the patch that shaded several plants during the day.

In early May 2007 when new foliage was just beginning to emerge, 36 individual kudzu plants, each with a rootcrown at the plant/soil interface, were located and surrounding vegetation was cleared. Woody, overwintered vines were removed by cutting so that only new growth would originate from the rootcrown. Each plant was enclosed in a cylindrical metal wire cage (1 m diameter, 0.92 m tall), lined on the inside with blue vinyl tarp material to prevent attack by mammalian herbivores and to confine plants to equal sized growing areas. The area inside cages was kept clear of competing vegetation by hand-weeding. Because plants within a kudzu patch can vary in age and resource availability, length and width measurements accurate to 0.005 cm were taken for each rootcrown using calipers (Scherr-Tumico Industries, Inc.; St. James, MN). The area of an ellipse ( $\pi * \frac{1}{2} \text{ length} * \frac{1}{2} \text{ width}$ ) was calculated to estimate the size of each rootcrown, for potential use as a covariate in analyses.

One of four treatments was applied to each plant, assigned randomly: cutting 50% of each leaflet of every leaf; clipping off the last 15 cm from 50% of the total number of shoots; drilling a hole from the rootcrown into roots, and a no-damage control ( $N = 9$  for each treatment). All treatments were applied to plants once per week over a 16-wk period from June to September. Leaf damage (cutting the right side of each leaflet without damaging the midvein) was applied to all leaves per plant in this treatment during the first week of study, and to new, fully expanded leaves each week thereafter. Leaf damage simulated herbivory by defoliating insects, the second largest feeding guild on the plant in China that produced about 13% damage (Sun et al., 2006). For the shoot clipping treatment, the length of all shoots was compared to a 15

cm ruler from the tip back toward the rootcrown, the total number of shoot tips over 15 cm counted, and the last 15 cm of 50% of the total number of shoot tips were removed by clipping. This treatment simulated the terminal-feeding guild reported in Sun et al. (2006), which was represented by a curculionid that produced similar damage when studied in quarantine in the US (Frye et al., 2007), and two species of attelabid beetles. Twenty to 40% damage was reported for terminal clippers in China, with up to 90% clipping found at some sites (Sun et al., 2006). The depth of drilling for the root treatment started at 0.02 cm in week one and increased by a factor of 1.3 to a total depth of 10.24 cm by week 16. The incremental increase, total depth, diameter of the drill bit (0.64 cm), and location of drilling from the rootcrown into roots were derived from information on a root-feeding cerambycid known to attack the plant in China (Sun et al., 2006). Above-ground plant biomass was harvested by cutting vines at the rootcrown one week after the last treatment application (week 17). Plant material was transported to the laboratory and stored at 2 °C. The number of stem nodes was counted for each plant, and plant material was placed in individually marked bags, dried in a greenhouse for several weeks and weighed (Ohaus Brainweigh B15000D; Pine Brook, NJ). Due to vandalism, the Dover site was abandoned after the 2007 field season, but plant survival was assessed in April 2008.

Two different field sites were used for the experiment in 2008 and 2009, Smyrna, DE (39° 14' 34.92" N, 75° 35' 13.22" W) and West Chester, PA (39° 55' 14.92" N, 75° 40' 41.56" W). Site characteristics of the two patches were dramatically different. The Smyrna site (Delaware/DE hereafter) is located between a four lane highway (US Route 13) and the Leipsic River. Rainwater flows down a steep bank from Route 13, onto the flat kudzu patch and into the river. The soil at this site is

sandy, with very little organic matter. Except for early morning hours, plants are in direct sunlight most of the day, and low- to medium-sized herbs are common at the site (bull thistle, *Cirsium vulgare* (Savi) Ten.; mugwort, *Artemisia vulgaris* L.; mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross; poison ivy, *Toxicodendron radicans* (L.) Kuntze). The site in West Chester (Pennsylvania/PA hereafter) is on an east-facing slope in a light gap surrounded by mesic woods, with a small stream at the base of the slope. The soil is characteristic of the Piedmont (McAvoy and Bennett, 2001), having a high organic content. Several invasive plants are abundant in the site, including multiflora rose (*Rosa multiflora* Thunb.), Japanese stiltgrass (*Microstegium vimineum* (Trin.) A. Camus), and mile-a-minute weed. Trees such as tulip tree (*Liriodendron tulipifera* L.) and black walnut (*J. nigra*) surround the patch on all sides and shade plants variably throughout the day. Although the exact history of the patches is not known, the observation that both are found on steep slopes adjacent to running water suggests that kudzu was intentionally planted on these sites for soil stabilization.

Following isolation of 36 plants at both the DE and PA field sites, woody above-ground biomass was removed, cages were constructed and rootcrown measurements were taken as described above. Treatments were randomly assigned and applied weekly to individual plants for 16 wk. Two levels of each damage type were investigated: cutting 50 and 75% of each leaflet per leaf; clipping off the last 15 cm from 50 and 75% of the total number of shoots; drilling one (Root 1) or two (Root 2) 10.24 cm deep holes from the rootcrown into the roots using the same incremental increase of 1.3 per week as described above; and a no-damage control ( $N = 9$  for the control, 5 for 75% damage levels and Root 2, and 4 for 50% damage levels and Root 1). Seventy-five percent leaf cutting was achieved by removing the right half of each

leaflet per leaf, and cutting the remaining leaflet in half so that only the lower left quarter of each leaflet remained intact. Above-ground biomass of all plants was harvested around the time of the first killing frost, the week of 19 October 2008. Plants from the DE site were transported and stored, and the number of stem nodes and dry weight were determined as above. Several plants from the PA site were damaged by an early frost, and it was not possible to count the number of stem nodes. Therefore, in 2008 only above-ground biomass was determined for surviving plants from PA.

In 2009 the study was repeated in DE and PA with the same plants. Overwintering survival to 2009 was recorded, and plants received the same damage treatments weekly for 16 wk. In October 2009, above-ground plant biomass was harvested and the total number of nodes and dry weight were determined for all surviving plants from both sites. All field plants were killed by cutting below the soil surface to remove the rootcrown. In both years, plants that did not survive were not included in statistical analysis.

### **North Carolina Field Experiment**

From 2007 to 2009 a field study was conducted at an established kudzu patch in Butner, North Carolina (36° 09' 58.09" N, 78° 47' 03.20" W). The site is adjacent to an agricultural field, was relatively flat and under full sun exposure. Twenty-four rootcrowns were isolated, measured (length and width to determine area of an ellipse) and designated as small (3.47 to 10.31 cm<sup>2</sup>), medium (13.33 to 25.72 cm<sup>2</sup>), or large (31.49 to 55.29 cm<sup>2</sup>). The three rootcrown size designations were replicated twice per treatment, and treatments included weekly cutting of 50% of each leaflet per leaf, removing 50% of the total number of vines greater than 15 cm by clipping at the base of the plant, drilling a 10.24 cm hole from the rootcrown into the

roots, and a no-damage control. Vine removal in NC differed from shoot damage in DE and PA, and represented the stem-feeding guild reported by Sun et al. (2006). In China, greater than 50% of vines were damaged by cerambycid and buprestid stem-boring beetles, and damage levels as high as 80% were reported at some sites (Sun et al., 2006). Leaf cutting and root drilling treatments were the same for all sites.

In 2007, cages made of clear polyethylene sheeting were erected around individual plants to prevent attack by mammalian herbivores. In 2008 and 2009 a single perimeter-style fence was used to exclude herbivores, and plants were kept isolated from surrounding vegetation by a combination of mowing and spot treatments with glyphosate. Treatments were applied to the same plants from 2007 to 2009 once per week for 16 wk. At the end of each season above-ground plant biomass was harvested, final stem node counts were made, and plants were dried for approximately 4 d in a gas-fired curing barn with strong air circulation at ~62.7 °C prior to weighing.

### **Greenhouse Experiment**

This experiment took place on three metal benches in a greenhouse room in Newark, DE with daily temperatures from 18 to 22 °C. Benches were oriented lengthwise from north to south, and a rectangular PVC frame (1 m tall) was placed on each bench. The frame on the west side of the room was covered with a dark cloth that extended from the top of the frame down to the bench, covering all sides except the north facing side to allow airflow. This design prevented direct light from reaching the plants, and was thus designated 0% direct light. A shade cloth that permitted 60% ambient light to reach plants was used for the middle bench, and again covered all sides except the north side. The third bench contained a frame only. Therefore, plants were either exposed to 0, 60, or 100% direct light.

Seeds for this study were collected in 2007 from Glasgow, DE (39° 37' 10.45" N, 75° 44' 43.89" W) and Montrose, NY (41° 14' 59.96" N, 73° 56' 17.72" W). Pods were hulled and seeds were stored at room temperature. For all seeds (~300 for NY, ~150 for DE), a portion of the seedcoat was removed by cutting with a scalpel to break coat-imposed seed dormancy (Susko et al., 2001), and seeds were sown in Pro-mix (Premier Horticulture; Red Hill, PA) in 7.6 cm<sup>2</sup> pots with 25 pots per tray. Two seeds were sown per pot for NY seeds on 25 February 2009, and one seed per pot for DE seed on 14 March 2009. Immediately after seeds were sown, two trays (total of 50 pots) from each seed source were placed on benches in one of the three light treatments. Pots were watered by hand as needed, and fertilized once per week with 200 ppm nitrogen solution. Of the total number of seeds planted, percent emergence (appearance of the shoot above the soil) was recorded on 7 April 2009 (41 d after planting for NY seed, 24 d after planting for DE seed), when seedlings from both seed sources were approximately the same size. The number of seedlings present on 14 April 2009, prior to leaf cutting, was used to determine percent survival or early season establishment weekly for the next 8 wk.

Treatments were assigned randomly to plants the week of 13 April 2009 by flipping a coin, and in each light treatment included 50% leaf cutting for DE seedlings, 75% leaf cutting for NY seedlings, and equal numbers of no-damage controls for both seed sources. Treatments were applied to all leaves the first week of study, and to newly expanded leaves thereafter. Leaf cutting treatments were never applied to cotyledons. Spinosad (Conserve SC, Dow AgroSciences LLC; Indianapolis, IN) was applied to all plants starting 16 April and once per week thereafter for control of thrips.

On 11 May 2009, individual pots were removed from trays and distributed evenly on benches to prevent overlap of foliage and potential shading. All above-ground biomass of plants was harvested the week of 20 July 2009 by cutting at the soil surface. Vine lengths were measured and the number of stem nodes per length of vine was counted. These data were used to calculate the average internode length for each plant. Above-ground biomass was determined for dried plant material as above. Plants that did not survive were not included in this analysis.

### **Statistical Analysis**

Statistical analyses were performed using SAS (SAS Institute, 2008). For field experiments, regression (PROC REG) was used to assess the relationship between rootcrown size and plant biomass. Analysis of variance (ANOVA) was used to compare rootcrown size, above-ground biomass, number of stem nodes by damage treatment for field studies with rootcrown size used as a covariate, and mortality by rootcrown size for the DE and PA sites. Two-way ANOVA was used to assess year by treatment interactions and year by site interactions for both biomass and the number of stem nodes in field studies. In the greenhouse, two-way ANOVA was used to assess biomass, vine length, internode length and the number of stem nodes for light by damage treatment interactions. The Shapiro-Wilk test in the UNIVARIATE procedure was used to test the assumption of normality, and Levene's test in the GLM procedure was used to test for homoscedasticity of the data. The natural log transformation was used to adjust data that violated one or both of these assumptions. If transformed data also violated ANOVA assumptions, the nonparametric Kruskal-Wallis test of the NPAR1WAY WILCOXON procedure was used. These data were ranked and an ANOVA was performed on the ranks. Tukey's test was used for mean separation. Chi-

square analysis was used to test plant survival for field and greenhouse studies. Greenhouse survival data was arcsine-squareroot transformed because the proportion of surviving plants was outside the range of 30-70% (Snedecor and Cochran, 1967).

## RESULTS

### Delaware and Pennsylvania Field Experiment

Prior to the start of the experiment in 2007 it was determined that rootcrown size did not differ by treatment in the pilot study ( $F_{3,30} = 0.40$ ,  $P = 0.7531$  for natural log transformed data). At the end of the season, rootcrown size was significantly correlated with above-ground biomass (Table 1.1), indicating that this measure could be used as a covariate for analysis to remove among-plant variation. Damage treatments in the pilot study did not affect the number of stem nodes ( $F_{4,29} = 3.90$ ,  $P = 0.3246$ ) or above-ground biomass ( $F_{4,29} = 13.84$ ,  $P = 0.3246$ ). In 2008, plant survival could not be assessed for all plants due to vandalism, but survival was at least 53% and possibly as high as 67%.

**Table 1.1.** Average crown size per site and relationship with above-ground biomass after the first year of study.

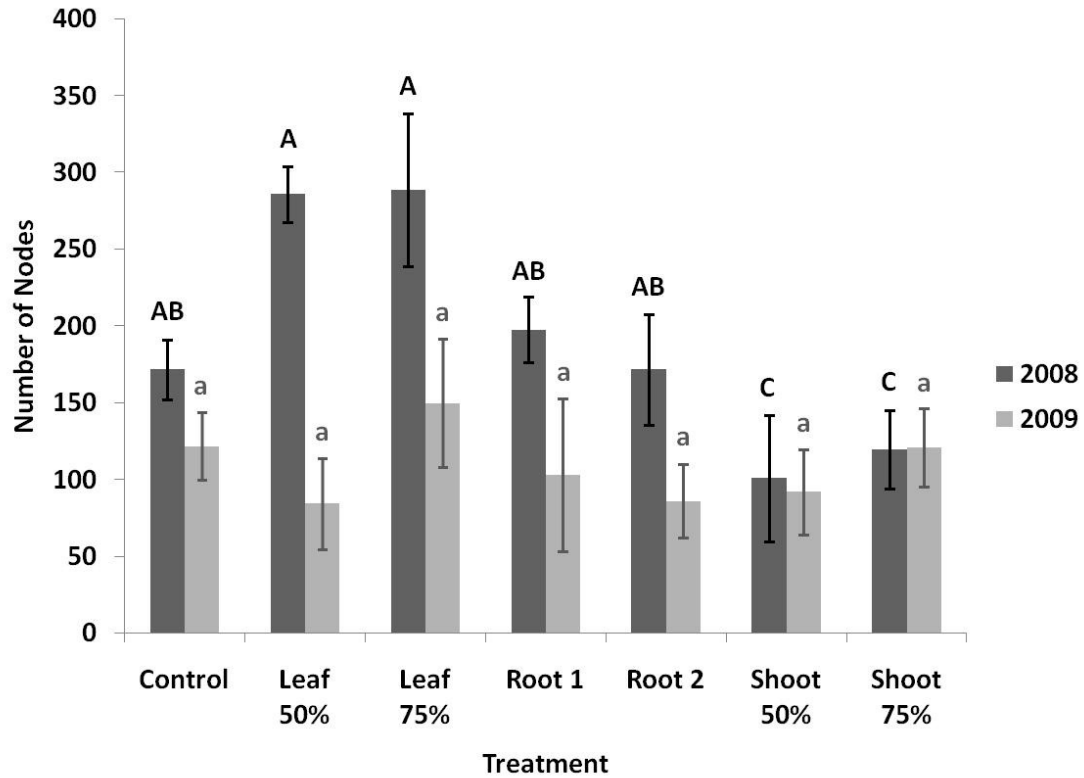
| Location (year initiated) | Crown Size <sup>a</sup> | N  | Above-ground biomass correlation |       |          |                |
|---------------------------|-------------------------|----|----------------------------------|-------|----------|----------------|
|                           |                         |    | F                                | df    | P        | r <sup>2</sup> |
| Pilot study (2007)        | 16.72 ± 3.46 B          | 34 | 53.35                            | 1, 32 | < 0.0001 | 0.6133         |
| DE (2008)                 | 30.58 ± 2.89 A          | 36 | 7.70                             | 1, 34 | 0.0089   | 0.1607         |
| NC (2007)                 | 22.53 ± 3.13 AB         | 24 | 0.86                             | 1, 22 | 0.3639   | -0.0061        |
| PA (2008)                 | 16.65 ± 2.33 B          | 36 | 4.30                             | 1, 34 | 0.0457   | 0.0863         |

<sup>a</sup> Means ± SEM with the same letter are not significantly different ( $F_{3,124} = 6.65$ ,  $P = 0.0003$  for natural log transformed data; Tukey's test used for mean separation).

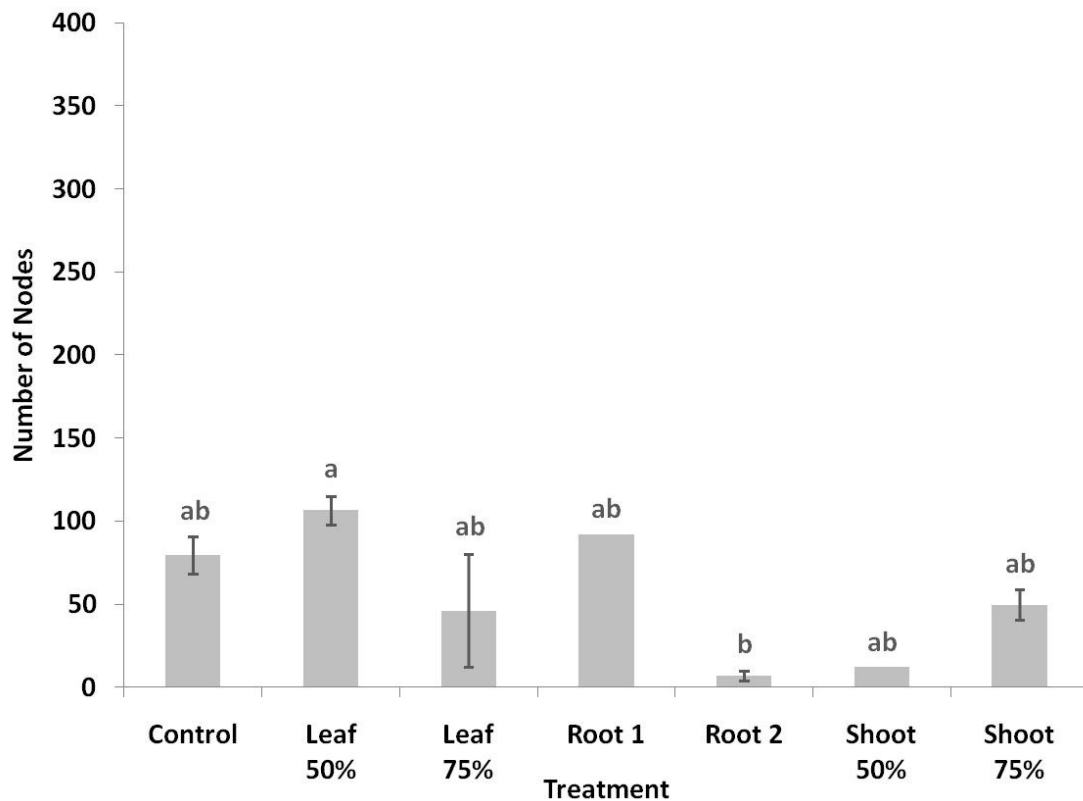
For field studies conducted at the two new sites in DE and PA in 2008, there was no difference in rootcrown size by treatment at either site (DE:  $F_{6,29} = 1.62$ ,  $P = 0.1763$ ; PA:  $F_{6,29} = 0.45$ ,  $P = 0.8371$  for natural log transformed data), and although  $r^2$  values were very low, rootcrown size was significantly correlated with above-ground biomass at the end of the first year (Table 1.1). Rootcrown sizes were significantly larger in DE than PA (Table 1.1), and ranged from 1.391 to 73.027 cm<sup>2</sup> in DE, and 1.397 to 51.244 cm<sup>2</sup> in PA.

In DE, survival to 2009 was 86%, and did not differ significantly by treatment ( $\chi^2_6 = 1.5295$ ,  $P = 0.9575$ ). Overall plant survival to 2009 in PA was low (50%), with only two plants in the Root 2 and both leaf cutting treatments, and one plant surviving in the Root 1 and Shoot 50% treatments. There was no significant difference in plant survival by treatment in PA ( $\chi^2_6 = 3.6811$ ,  $P = 0.7197$ ). When sites were compared, mortality was significantly higher in PA than DE ( $\chi^2_1 = 7.3478$ ,  $P = 0.0067$ ), and pooled data from both sites showed that plants with smaller rootcrowns had higher mortality (died:  $16.44 \pm 2.72$  cm<sup>2</sup>; survived:  $26.67 \pm 2.53$  cm<sup>2</sup>;  $F_{1,70} = 6.10$ ,  $P = 0.0160$ ).

There were no differences in the number of stem nodes between the control, leaf and root damage treatments for DE plants in 2008, but shoot clipping significantly reduced stem node numbers compared to all other treatments ( $F_{7,28} = 5.47$ ,  $P = 0.0007$ ; Figure 1.1). In 2009, there was no difference in the number of stem nodes for any damage treatment in DE ( $F_{7,23} = 0.53$ ,  $P = 0.7793$ ; Figure 1.1), and although there were significant differences for plants in PA ( $F_{7,10} = 4.07$ ,  $P = 0.0251$ ), there was no distinct pattern by treatment (Figure 1.2).



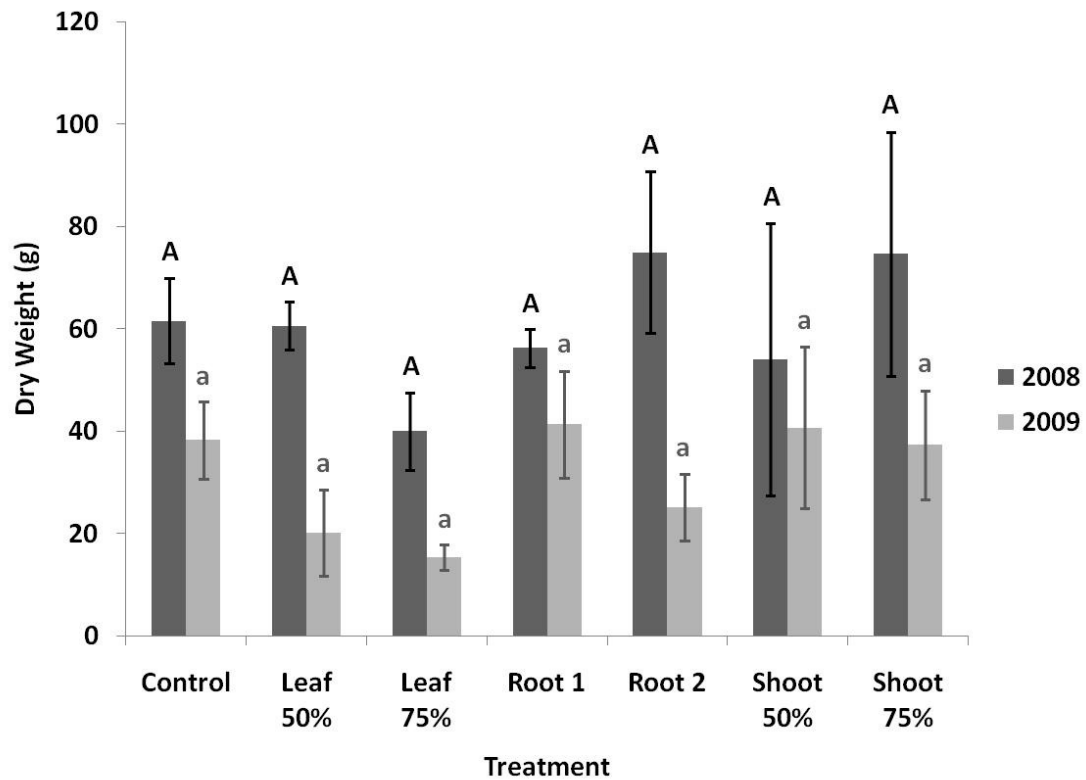
**Figure 1.1.** Average number of stem nodes for DE plants at the end of the 2008 and 2009 seasons. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{7,28} = 5.47$ ,  $P = 0.0007$ ,  $N = 9$  for Control; 5 for Leaf 75%, Root 2, Shoot 75%; 4 for Leaf 50%, Root 1, Shoot 50%; 2009:  $F_{7,23} = 2.10$ ,  $P = 0.0930$  for natural log transformed data,  $N = 9$  for Control; 5 for Shoot 75%; 4 for Leaf 50%, Root 2, Shoot 50%; 3 for Leaf 75%; 2 for Root 1; Tukey's test used for mean separation).



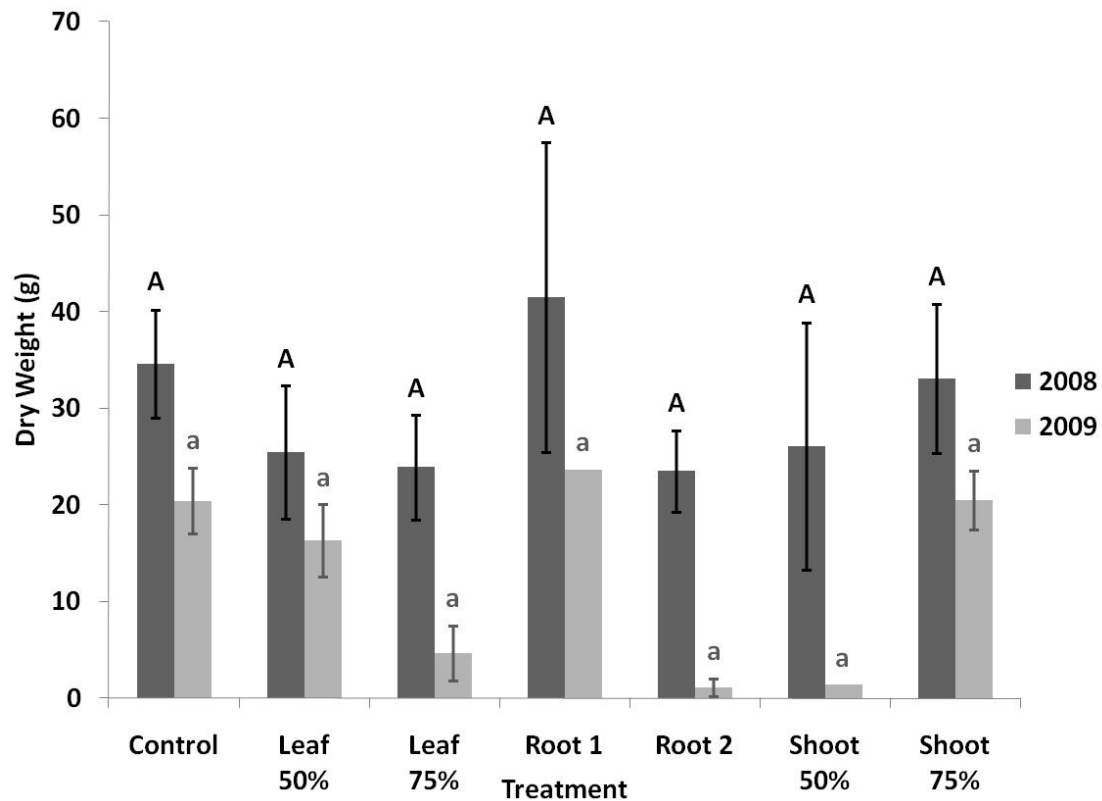
**Figure 1.2.** Average number of stem nodes for PA plants at the end of the 2009 season. Means ( $\pm$  SEM) with the same letter are not significantly different ( $F_{7, 10} = 4.07$ ,  $P = 0.0251$ ,  $N = 5$  for Control, Shoot 75%; 2 for Leaf 50%, Leaf 75%, Root 2; 1 for Root 1, Shoot 50%; Tukey's test used for mean separation). Data for 2008 not available.

Above-ground biomass of plants from DE (Figure 1.3) and PA (Figure 1.4) did not differ significantly by treatment in 2008 or 2009. However, average above-ground biomass per site was significantly higher in DE ( $60.7 \pm 5.33$  g,  $N = 36$ ) compared to PA in 2008 ( $30.4 \pm 2.98$  g,  $N = 34$ ;  $F_{13, 56} = 20.61$ ,  $P < 0.0001$ ). In 2009, biomass was again higher in DE ( $32.4 \pm 3.80$  g,  $N = 31$ ) than in PA ( $15.2 \pm 2.30$ ,  $N = 18$ ;  $F_{13, 35} = 9.04$ ,  $P = 0.0049$ ), despite significant reductions in biomass for plants at

both sites in 2009 compared to 2008 ( $F_{3,115} = 27.78$ ,  $P < 0.0001$  for natural log transformed data).



**Figure 1.3.** Above-ground biomass of DE plants in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{6,29} = 0.70$ ,  $P = 0.6522$  for ranked data,  $N = 9$  for Control; 5 for Leaf 75%, Root 2, Shoot 75%; 4 for Leaf 50%, Root 1, Shoot 50%; 2009:  $F_{7,23} = 0.78$ ,  $P = 0.5771$  for natural log transformed data,  $N = 9$  for Control; 5 for Shoot 75%; 4 for Leaf 50%, Root 2, Shoot 50%; 3 for Leaf 75%; 2 for Root 1; Tukey's test used for mean separation).



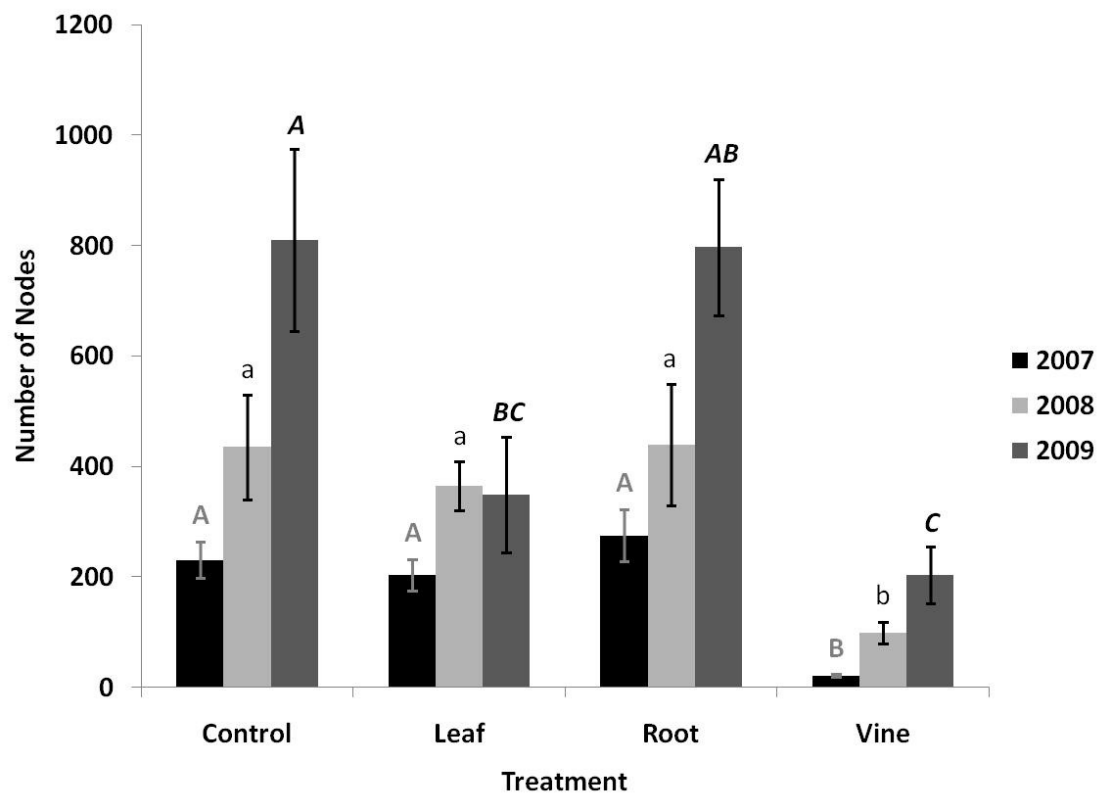
**Figure 1.4.** Above-ground biomass of PA plants in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{6,27} = 0.44$ ,  $P = 0.8480$  for ranked data,  $N = 9$  for Control; 5 for Leaf 75%, Root 2, Shoot 75%; 4 for Root 1; 3 for Leaf 50%, Shoot 50%; 2009:  $F_{7,10} = 4.04$ ,  $P = 0.0256$ ,  $N = 5$  for Control, Shoot 75%; 2 for Leaf 50%, Leaf 75%, Root 2; 1 for Root 1, Shoot 50%; Tukey's test used for mean separation).

### North Carolina Field Experiment

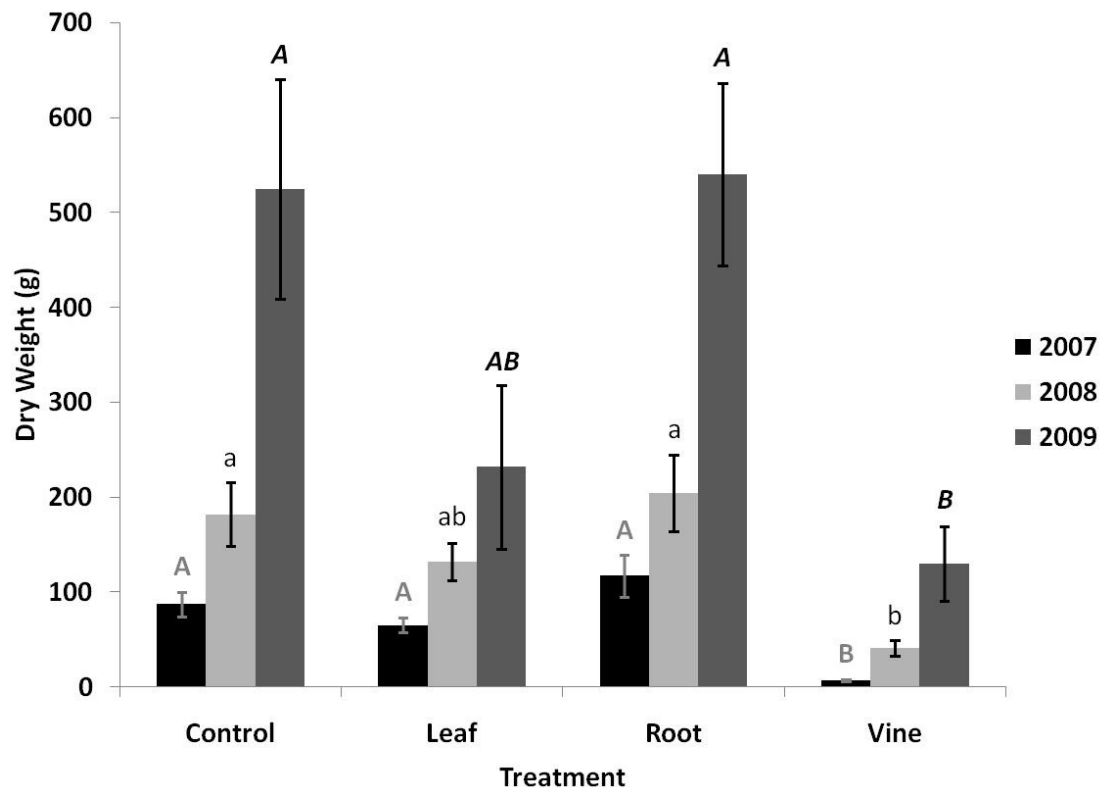
In NC, the method of assigning treatments to plants differed from DE and PA, but when analyzed by treatment there was no difference in rootcrown size ( $F_{3,20} = 0.03$ ,  $P = 0.9913$ ). Rootcrown size in NC ranged from 3.470 to 55.288 cm<sup>2</sup>, and the average rootcrown size of plants was intermediate between values obtained for DE and

PA (Table 1.1). At the end of the first season, rootcrown size was not significantly correlated with above-ground biomass (Table 1.1).

Plant survival in NC from 2007 to 2009 was 100%. Vine removal significantly reduced the number of stem nodes compared to the control and root drilling treatments in all years (Figure 1.5). In 2009, leaf and vine damage reduced stem node numbers relative to the control (Figure 1.5). Above-ground biomass was not affected by leaf cutting or root drilling compared to the control in any year (Figure 1.6). However, vine removal significantly reduced biomass compared to both the control and root drilling treatments from 2007 to 2009 (Figure 1.6). Plant biomass was significantly affected by year ( $F_{11,60} = 30.33, P < 0.0001$ ) and damage treatment ( $F_{11,60} = 11.59, P < 0.0001$ ) in NC. However, a significant interaction term between year and treatment ( $F_{11,60} = 2.73, P = 0.0207$ ) precludes further analysis of these individual effects.



**Figure 1.5.** Average number of stem nodes for NC plants from 2007 to 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2007:  $F_{3,20} = 9.76$ ,  $P = 0.0004$  for ranked data; 2008:  $F_{4,19} = 14.39$ ,  $P < 0.0001$  for natural log transformed data; 2009:  $F_{4,19} = 5.87$ ,  $P = 0.0057$ ;  $N = 6$  for all treatments and years; Tukey's test used for mean separation).



**Figure 1.6.** Above-ground biomass of NC plants from 2007 to 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2007:  $F_{3,20} = 14.65$ ,  $P < 0.0001$  for ranked data; 2008:  $F_{4,19} = 8.87$ ,  $P = 0.0007$ ; 2009:  $F_{4,19} = 7.60$ ,  $P = 0.0015$ ;  $N = 6$  for all treatments and years; Tukey's test used for mean separation).

### Greenhouse Experiment

Cutting the seedcoat resulted in seedling emergence from soil 5-6 d after planting. Percent emergence for the 0, 60 and 100% light treatments was 23, 24, and 21% for NY seed ( $N = 100$  for each light treatment), and 88, 88, and 94% for DE seed ( $N = 50$  for each light treatment). Although percentages were low for NY seed, these values suggest that light environment did not affect seedling emergence from either seed source. However, casual observations indicated that plants in the 0% light

treatment were etiolated, had darker leaves than plants in either the 60 or 100% light treatments, and stopped growth at approximately 5 cm. There were no obvious differences between plants in 60 and 100% direct light based on these casual observations.

For NY seedlings, plants in 0% direct light did not survive after 12 May (76 d after planting), regardless of whether leaves were cut. At the end of the 10 wk experiment, the overall survival for NY seedlings was 53%, and no differences were found in the proportion of surviving plants by treatment (Table 1.2). Overall survival of DE seedlings was 65%. There were no differences in the proportion of plants surviving to the end of the season by treatment, but all control plants in 60 and 100% direct light survived (Table 1.3). A single control plant in 0% direct light survived, but was not included in subsequent analysis since all other DE plants in 0% light had died by 2 June (80 d after planting).

**Table 1.2.** New York plant response to light and cutting treatments. Means  $\pm$  SEM with the same letter are not significantly different ( $P > 0.05$ , ANOVA, Tukey's test used for mean separation).

| <b>Treatment</b>    | <b><i>N</i><br/>(% survival)<sup>a</sup></b> | <b>Vine length (cm)</b> | <b>Internode<br/>distance (cm)</b> | <b>Number of<br/>stem nodes</b> | <b>Above-ground<br/>biomass (g)</b> |
|---------------------|--|-------------------------|------------------------------------|---------------------------------|-------------------------------------|
| 100% Light Control  | 8 (100)                                      | 80.88 $\pm$ 10.85 A     | 2.58 $\pm$ 0.31 A                  | 30.88 $\pm$ 1.51 AB             | 5.94 $\pm$ 0.35 A                   |
| 100% Light Leaf 75% | 7 (70)                                       | 30.86 $\pm$ 10.47 BC    | 0.68 $\pm$ 0.20 B                  | 40.57 $\pm$ 6.31 A              | 2.66 $\pm$ 0.60 BC                  |
| 60% Light Control   | 6 (60)                                       | 45.17 $\pm$ 7.69 AB     | 2.51 $\pm$ 0.55 A                  | 19.50 $\pm$ 1.52 B              | 2.92 $\pm$ 0.19 B                   |
| 60% Light Leaf 75%  | 7 (64)                                       | 15.00 $\pm$ 5.20 C      | 0.62 $\pm$ 0.09 B                  | 21.14 $\pm$ 5.69 AB             | 1.06 $\pm$ 0.44 C                   |
| 0% Light Control    | 0 (0)  | -----                   | -----                              | -----                           | -----                               |
| 0% Light Leaf 75%   | 0 (0)  | -----                   | -----                              | -----                           | -----                               |

<sup>a</sup> *N* represents the number of living plants per treatment at the end of the experiment, used for measurements. Percent survival was determined from the starting number of plants per treatment on 14 April. No differences were detected in percent survival of plants by treatment ( $\chi^2_1 = 0.0657$ ,  $P = 0.7977$ ).

**Table 1.3.** Delaware plant response to light and cutting treatments. Means  $\pm$  SEM with the same letter are not significantly different ( $P > 0.05$ , ANOVA, Tukey's test used for mean separation).

| <b>Treatment</b>    | <b><i>N</i><br/>(% survival)<sup>a</sup></b> | <b>Vine length (cm)</b> | <b>Internode<br/>distance (cm)</b> | <b>Number of<br/>stem nodes</b> | <b>Above-ground<br/>biomass (g)</b> |
|---------------------|--|-------------------------|------------------------------------|---------------------------------|-------------------------------------|
| 100% Light Control  | 24 (100)                                     | 93.13 $\pm$ 6.36 A      | 2.71 $\pm$ 0.20 B                  | 35.0 $\pm$ 1.18 B               | 5.84 $\pm$ 0.26 A                   |
| 100% Light Leaf 50% | 19 (95)                                      | 87.74 $\pm$ 8.04 A      | 1.98 $\pm$ 0.17 B                  | 44.1 $\pm$ 1.80 A               | 5.72 $\pm$ 0.28 A                   |
| 60% Light Control   | 22 (100)                                     | 89.14 $\pm$ 7.32 A      | 3.60 $\pm$ 0.28 A                  | 25.2 $\pm$ 1.76 C               | 4.41 $\pm$ 0.25 B                   |
| 60% Light Leaf 50%  | 17 (77)                                      | 72.71 $\pm$ 6.65 A      | 2.56 $\pm$ 0.26 B                  | 29.5 $\pm$ 1.82 BC              | 3.48 $\pm$ 0.19 B                   |
| 0% Light Control    | 1 (5)  | 7.00 $\pm$ 0.00         | 1.17 $\pm$ 0.00                    | 6.0 $\pm$ 0.00                  | 0.20 $\pm$ 0.00                     |
| 0% Light Leaf 50%   | 0 (0)  | -----                   | -----                              | -----                           | -----                               |

<sup>a</sup> *N* represents the number of living plants per treatment at the end of the experiment used for measurements. Percent survival was determined from the starting number of plants per treatment on 14 April. No differences were detected in percent survival of plants by treatment ( $\chi^2_2 = 0.1840$ ,  $P = 0.9121$ ).

For both NY and DE seedlings, there were no significant light by cutting treatment interactions for any plant growth parameters measured ( $P > 0.05$ , 2-way ANOVA). Within each light treatment for NY seedlings, 75% leaf cutting significantly reduced total vine length ( $F_{3,24} = 10.04$ ,  $P = 0.0002$  for ranked data), average internode length ( $F_{3,24} = 13.36$ ,  $P < 0.0001$  for ranked data), and above-ground biomass ( $F_{3,24} = 23.99$ ,  $P < 0.0001$ ) compared to control plants (Table 1.2). Furthermore, cut plants in 60% light had significantly reduced vine lengths and above-ground biomass than control plants in either light treatment (Table 1.2). Cut plants in 100% light produced significantly more stem nodes than control plants in 60% light ( $F_{3,24} = 4.68$ ,  $P = 0.0104$  for ranked data; Table 1.2).

There was no difference by treatment in total vine length for DE seedlings ( $F_{3,78} = 2.08$ ,  $P = 0.1097$ ; Table 1.3). Average internode length for DE seedlings was longest for control plants in 60% light compared to all other treatments ( $F_{3,78} = 8.39$ ,  $P < 0.0001$ ), and plants in 100% direct light always produced more above-ground biomass than plants in 60% light ( $F_{3,78} = 18.26$ ,  $P < 0.0001$ ; Table 1.3). Cut plants in 100% light produced the highest number of stem nodes compared to all other treatments ( $F_{3,78} = 24.41$ ,  $P < 0.0001$ ; Table 1.3).

## DISCUSSION

The goal of the current study was to document the response of individual plants in established kudzu patches to different types and levels of simulated herbivore damage near the center (North Carolina) and edge (Delaware and Pennsylvania) of kudzu's invasive range. For both DE and PA, we found that damage treatments did not affect above-ground biomass of plants, but complete vine clipping in NC significantly

and consistently reduced biomass compared to the control. Plant survival over two seasons at the edge of kudzu's range was 86 and 50% in DE and PA, compared to 100% survival in NC over three seasons. In the greenhouse, DE plants were able to compensate for 50% leaf damage in 60 and 100% direct light, but NY plants were unable to overcome 75% leaf damage, which significantly reduced biomass, vine length and internode length compared to the control within each light treatment. Although emergence was similar across light environments, only one of 53 plants survived to the end of the experiment when grown in 0% direct light. Finally, stem node counts appear to be of limited value in understanding plant response to damage, since no consistent relationships were recognized in DE, PA, or the greenhouse, while counts in NC provided no additional information to biomass data.

Kudzu is a perennial vine that spreads primarily by vegetative reproduction in the US. Vines in contact with soil produce adventitious roots at stem nodes, and can form physiologically independent plants in subsequent years when connections between nodes senesce (Forseth and Innis, 2004, and references therein). However, not much is known about the growth of plants formed by vegetative reproduction, particularly how resources are allocated among plant parts. Of particular interest in our study was the size of the rootcrown, or the structure at the interface of above- and below-ground plant parts. This structure is thought to vary in size based on plant age, resource availability, or herbivore attack. For example, *Cirsium altissimum* (L.) Spreng (Asteraceae) is a perennial herbaceous thistle that produces a basal rosette or rootcrown (Guretzky and Louda, 1997). When herbivores are excluded from the plant with insecticides, rootcrown size increases incrementally throughout the season. However, plants subject to herbivory by multiple herbivores produce less above-

ground biomass and also have a reduced crown size (Guretzky and Louda, 1997). At the end of the first year in the present study, we found a weak correlation between rootcrown size and above-ground biomass production, indicating that rootcrown size is a measure of plant potential productivity for a given year, and reflects the actual productivity of plants during the previous year. This makes intuitive sense, as kudzu is known to allocate carbohydrates to roots at the end of the growing season (Wechsler, 1977), and increases in the size of the rootcrown may be coupled with root expansion in the fall. Thus, to remove among-plant variation within a site, rootcrown size was included as a covariate in analyses of plant growth parameters.

For plants in DE and PA, there were no differences in biomass production in 2008 or 2009. One explanation for this result is plant compensatory response to herbivory, which has three possible outcomes for plants (Maschinski and Whitham, 1989). The first is under-compensation, which occurs when plants are unable to replace tissue or resume equal productivity based on losses to herbivores, and is often detrimental to plants. Complete compensation is when plants adjust productivity such that there are no differences in biomass or seed production compared to undamaged controls, and over-compensation is described as elevated biomass or seed production for plants subject to herbivory compared to control plants. Over-compensation occurs when plants overestimate the damage caused by herbivores (Maschinski and Whitham, 1989). In the current experiment, the observed lack of differences by treatment provides evidence that kudzu was able to completely compensate for tissue lost to simulated damage treatments. While interesting, this result was not unexpected because kudzu has a high allocation of resources to above-ground biomass (Forseth and Innis, 2004), and carbohydrates stored in large, tuberous roots can be used for

plant growth (Forseth and Teramura, 1986). For example, research reported elsewhere (Chapter 2) found root:shoot ratios close to, or below a value of one for kudzu plants subject to simulated leaf damage, indicating that resource allocation to above-ground plant parts occurs at the expense of root reserves. Thus, use of stored root resources by plants in DE and PA could overcome differences in biomass removal due to the different damage treatments and levels.

Although statistical comparisons cannot be made between plants grown near the center (NC) and edge (DE/PA) of kudzu's invasive range because of differences in damage treatments and no or limited site replication, it is interesting to note that plant biomass decreased after one year in DE and PA, but increased each year in NC. In previous studies with kudzu, it has been shown that plants in the shade produce less biomass and allocate fewer resources to roots than plants in full sun (Abramovitz, 1983; Fujita et al., 1993; Wechsler, 1977). In part, the results from our greenhouse experiment confirm these findings, as biomass of control plants grown in 60% direct light was reduced compared to plants in 100% light, and reduced biomass production would result in reduced resource allocation to roots. At the center and edge of kudzu's invasive range, one difference in our study that could have affected light exposure and biomass production was the use and design of structures to prevent attack from mammalian herbivores. In DE and PA, cylindrical cages with blue tarp material were used on east-facing slopes, while clear polyethylene cages in NC were used in year one, and no cages were used in subsequent years in a flat, open area. If plants in DE and PA experienced reduced light reception due either to environmental factors (natural shade) or experimental design (cage structure), photosynthesis and allocation to root reserves during the season would also be reduced for these plants.

This, in addition to a longer growing season in NC, probably explains why biomass in subsequent years increased for plants in NC and decreased in DE and PA.

Early growth by kudzu plants in the spring is derived from root reserves (Wechsler, 1977) as the plant is late to leaf out, yet has a high light requirement. Similarly, plants that have limited access to light will expend stored resources throughout the season to enhance vine growth and acquire additional light for photosynthesis (Forseth and Teramura, 1986). If plants from DE and PA were limited in their capacity to photosynthesize, then above-ground biomass produced in year one would be derived solely from stored root carbohydrates. In year two, the inability of plants to photosynthesize would further drain root reserves for above-ground biomass production. Because biomass production on average, and presumably the amount of stored carbohydrates in roots, was lower for plants in PA, total depletion of root reserves provides a compelling explanation for higher mortality of plants at this site. This result lends support to the commonly cited observation that repeated removal of above-ground biomass, whether by burning, herbicide application (Harrington et al., 2003), grazing by goats (Bonsi et al., 1991), mowing (Everest et al., 1991) or solarization (Newton et al., 2008) can control kudzu by exhausting stored carbohydrates of the plant.

Plants in NC, on the other hand, were exposed to full sunlight in all years, even when caged. Higher productivity of these plants due to enhanced photosynthesis suggests increased allocation of carbohydrates to roots at the end of the each season, providing additional resources for growth the following year. Full access to light therefore explains why plants in NC showed 100% survival over a three year period,

while plants in DE and PA had 86% and 50% survival after one year of growth in dark cages.

Considering the above information about foliar damage depleting roots and leading to plant mortality, the lack of effects by root drilling reported here is surprising. Research in other systems has shown that simulating root damage is difficult (Blossey and Hunt-Joshi, 2003), and our study of field kudzu plants was no different. Although we attempted to simulate burrowing of insects from the rootcrown into roots as observed for natural enemies in China (Sun et al., 2006), it was not always possible to drill directly into roots, and damage in several cases could more accurately be described as rootcrown damage. Furthermore, because treatments were assigned randomly, some plants had few, or small roots extending from the rootcrown into the soil at the start of the experiment, and drilling severed the connection between the rootcrown and roots for two plants in PA. As roots alone cannot regenerate above-ground plant parts without the meristematic tissue present in rootcrowns, this resulted in death of the two plants. In the field, this result may be unrealistic as reports from the China show that infested roots fall into two size categories, with average root diameters of 3.3 and 6.7 cm (Sun et al., 2006). Thus, small roots such as those described here would not be infested with herbivores in China, and root-feeding herbivores are not expected to kill their host plant. For potential root-feeding herbivores, it is evident that more research is needed to develop techniques of applying root damage in the field, and to estimate the impact this might have on individual kudzu plants.

Finally, a common misconception regarding the invasiveness of kudzu is that this plant is not capable of producing viable seed in the US (Mitich, 2000). While

seedcoat dormancy in general is known for leguminous plant species (Quinlivan, 1971), the results from our greenhouse study confirm that recruitment of kudzu seedlings is limited at least in part by low light availability (Abramovitz, 1983). Percent emergence of seeds from both NY and DE was relatively even among light treatments, but plant survival and establishment in 0% direct light was only 2%. In natural situations, shading can occur when native vegetation emerges early in the season and outcompetes kudzu seedlings for light, or more commonly when the canopy of an established kudzu patch shades the soil surface (Wechsler, 1977). At research sites in DE and PA, seedlings have been observed mid-summer in areas where the soil is disturbed, specifically on paths made to access plants, as well as within cages (personal observation). This has important implications for management, as removal of current kudzu plants with herbicides or by other control methods could result in recruitment of kudzu seedlings, or other exotic invasive species as part of the “invasive species treadmill” (Thomas and Reid, 2007).

In other systems, simulated herbivory experiments have been used to predict control efficacy of plants under different light exposures. For the rangeland weed, *Bromus tectorum* L., greenhouse studies demonstrated that the ability of plants to re-grow after simulated herbivore grazing was significantly reduced when plants were grown in the shade (Pierson et al., 1990). More recently, Milbrath (2008) demonstrated that artificial defoliation of *Vincetoxicum* sp. was effective at killing plants in the shade, but not in the sun. In the current study, we have identified a potential relationship between plant productivity, resource allocation and survival in established kudzu patches. Plants at the edge of kudzu’s invasive range that started with fewer resources, as measured by the size of the rootcrown, produced less above-

ground biomass and experienced higher mortality than plants with full access to sunlight near the center of kudzu's invasive range, regardless of damage treatments. Taken together with results from the greenhouse study, these experiments suggest that biological control of kudzu may be enhanced in low light environments compared to patches that are in full sun. In full sun environments, high levels of leaf damage or vine removal throughout the season may suppress plant growth and deplete root reserves. Therefore, we recommend that future studies in the plant's native range focus on the 37 leaf-feeding species and the 14 stem-feeding species identified by Sun et al. (2006) in the search for potentially host specific natural enemies of kudzu.

## **ACKNOWLEDGEMENTS**

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## Chapter 2

# RESPONSE OF KUDZU (*PUERARIA MONTANA* VAR. *LOBATA*) TO DIFFERENT TYPES AND LEVELS OF SIMULATED INSECT HERBIVORE DAMAGE

## INTRODUCTION

Kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & S. M. Almeida ex Sanjappa & Predeep (Suddath) (Fabaceae), is native to Asia and was intentionally introduced to the United States in 1876 at the Centennial Exposition in Philadelphia (Mitich, 2000). It was extensively planted for erosion control along rights of way in the 1930s and 1940s by the Civilian Conservation Corps (Miller and Edwards, 1983), and throughout the southeastern US on farmland for use as a high-nitrogen forage crop (Britton et al., 2002). Today, kudzu is listed as a top 10 invasive species worldwide (Friedman, 2010), and has recently been implicated in increases in ozone pollution (Hickman et al., 2010).

Kudzu is a perennial, woody vine that exhibits high allocation of resources to photosynthetic surfaces (Forseth and Innis, 2004). Growth rates of 10 to 30 m per vine have been reported for one season, and plant densities in infested areas are estimated at tens of thousands of individuals per hectare (Mitich, 2000). High densities result from the plant's ability to form adventitious roots where stem nodes contact the soil surface, and each rooted stem node can potentially form a new, freestanding plant the next year. On woody vines, meristem tissue in stem nodes allows vines to grow

higher into the tree canopy each year. In time, kudzu can blanket photosynthetic surfaces of native vegetation and ultimately kill trees, starting with those on the forest edge (Munger, 2002; Wechsler, 1977). Vine growth causes economic damage by interfering with the lumber and pulpwood industries, as well as costs to keep the vine off of rights of way and power lines (Shurtleff and Aoyagi, 1985). Current estimates of kudzu cover in the US are between two and seven million acres (Britton et al., 2002), and under current climate models a range expansion is expected, particularly in the Northeast (Jarnevich and Stohlgren, 2009; Weltzin et al., 2003).

In 1970 kudzu was classified as a common weed, yet large scale control of the plant remains a challenge. Mechanical removal of roots is difficult and expensive, and chemical applications are generally ineffective unless combined with other control methods (Harrington et al., 2003; Thomas, 2000). Permanent control requires eradication, since even a single plant can re-infest an area (Miller and Edwards, 1983). Because of its ecological and economic impacts, the USDA Forest Service targeted kudzu for classical biological control in 1999 (Britton et al., 2002). Surveys for natural enemies were conducted in the plant's native range, and over 100 phytophagous insects in six different feeding guilds were found on the plant in China (Sun et al., 2006). Two of these insects were evaluated for their host specificity in a US quarantine facility: a leaf-feeding beetle *Gonioctena tredecimmaculata* (Jacoby) (Coleoptera: Chrysomelidae) and a stem/terminal-feeding weevil *Ornatalcides (Mesalcidodes) trifidus* (Pascoe) (Coleoptera: Curculionidae). However, both insects were rejected as potential biological control agents due to feeding on soybean (*Glycine max* (L.) Merr.) and the native woodland plant hog-peanut (*Amphicarpaea bracteata* (L.) Fernald) (Frye et al., 2007).

Retrospective analyses of classical weed biological control programs have shown that the greatest risk of nontarget attack is for plants closely related to the target weed (Pemberton, 2000). Thus, unequivocal evidence from prerelease host specificity evaluations will be necessary for potential biological control agents of kudzu, since this weed is closely related to the agriculturally important crop soybean (Frye et al., 2007). To date, laboratory no-choice tests in quarantine that describe an insect's fundamental host range are considered the best method of determining safety prior to release (Marohasy, 1998). However, this process can be expensive, requiring up to 3 scientist years and over \$460,000 per agent (McFadyen, 1998), yet positive outcomes such as the identification of a safe natural enemy are not guaranteed (e.g., Frye et al., 2007; McFadyen and Weggler-Beaton, 2000; Wang et al., 2010). Alternatively, an approach that puts "the filter of efficacy before safety" (Raghu and Dhileepan, 2005) is the use of simulated herbivory studies to refine the number of prospective biological control agents in the native range to only those in feeding guilds predicted to impact the target weed (Raghu et al., 2006), and does so by documenting plant response to different types and levels of damage in the introduced range (Wirf, 2006).

The current study was undertaken to document the response of newly established kudzu plants to different types and levels of simulated herbivore damage in an open, agricultural setting near the center (North Carolina) and edge (Delaware) of the plant's invasive range. Along with a companion study conducted on plants in established kudzu patches in Delaware, North Carolina, and Pennsylvania (Chapter 1), this detailed information can provide a framework to prioritize potential insect feeding guilds of biological control agents, and reduce the costs of studying ineffective or unsafe natural enemies in quarantine.

## MATERIALS AND METHODS

### Delaware Experiment

#### *Seed collection, emergence, and herbicide selection*

A non-replicated study was completed to identify a seed source (kudzu patch) that had the greatest number of fully developed seeds per pod. Seedpods were collected from the following five sites in the fall 2007: Dover, DE (39° 10' 56.97" N, 75° 32' 46.76" W); Glasgow, DE (39° 37' 10.45" N, 75° 44' 43.89" W); Montrose, NY (41° 14' 59.96" N, 73° 56' 17.72" W); Smyrna, DE (39° 14' 34.92" N, 75° 35' 13.22" W); and West Chester, PA (39° 55' 14.92" N, 75° 40' 41.56" W). For a random sample of 50 pods per location, pod length was measured and the number of fully developed seeds, as distinguished from aborted seeds, was counted for each pod. Subsequently, an efficacy trial to determine the best method for breaking coat-imposed seed dormancy was completed with seed from Glasgow, DE and Montrose, NY. Treatments were applied to 50 seeds per location, based on Susko et al. (1999, 2001) and included abrasion with 120 grit sandpaper, boiling in distilled water for 10 s, placing seeds in a dry heat oven at 100 °C for 10 min, soaking in 100% ethanol for 12 hrs, and use of a scalpel to either nick or remove a portion of the seedcoat (two separate treatments; Susko et al., 1999). Seeds soaked in ethyl alcohol were rinsed with distilled water, and along with boiled seeds were set to dry on filter paper before planting. All seeds were planted individually 4 mm deep (Susko et al., 1999) in 50-cell flats, and each 5 cm square cell contained Redi-earth (Sun Gro Horticulture Canada Ltd.; Vancouver, British Columbia). Flats were placed in a greenhouse mist-room that waters plants for one minute six times in a 12 hour period. Percent emergence and the total number of

seedlings per treatment that survived to the two leaf stage (18 d after planting) were recorded, and seedlings were transferred individually to 15-cm diameter round pots with Pro-mix (Premier Horticulture; Red Hill, PA), watered twice daily by hand, fertilized once per week with a 200 ppm nitrogen solution, and grown in a temperature and humidity controlled greenhouse room.

When plants reached the six leaf stage, they were used for a trial to evaluate the effects of different herbicides on kudzu growth. The purpose of this investigation was to determine which herbicides could be used to suppress the growth of agricultural weeds, but not affect the growth of experimental kudzu plants in the field. The trial was conducted in June 2008 at the University of Delaware Research and Education Center in Georgetown, DE. Three to four plants from each seed source (Glasgow, DE; Montrose, NY) were lined up and evenly spaced in an agricultural field and the following herbicides, formulated at twice the normal concentration, were sprayed on plants at a rate of 236.5 L per hectare at 4.8 kilometers per hour with 1.5 Kg per cm pressure from a backpack sprayer: atrazine (Atrazine 4L, Syngenta Crop Protection, Inc.; Greensboro, NC), mesotrione (Callisto, Syngenta Crop Protection, Inc.), chlorimuron (Classic, DuPont Crop Protection; Wilmington, DE), cloransulam (FirstRate, Dow AgroSciences LLC; Indianapolis, IN), imazethapyr (Pursuit, BASF Corporation; Florham Park, NJ), fomesafen (Reflex, Syngenta Crop Protection, Inc.), and halosulfuron-methyl (Sanda, Gowan Company LLC; Yuma, AZ). After herbicide application, plants were transported to a greenhouse in Newark, DE, monitored over the next 12 days, and damage (leaf burn, bleaching, chlorosis, etc.) was recorded.

### ***Farm experiment***

This study took place on a 28 by 41 m farm field in Newark, DE. Site preparation included tillage and application of 1.5 L per hectare of *S*-metolachlor (Dual Magnum, Syngenta Crop Protection, Inc.) and 0.15 L per hectare of imazethapyr as preemergence herbicides (Mark VanGessel, University of Delaware, personal communication). The plot was divided into seven rows, each containing ten 1 by 5 m plots (70 total). Plots in a row were separated by 2 m alleys, and a 1 m alley separated rows. Plots in each row were grouped into two blocks of five adjacent plants. One block consisted of 1-year plants, or plants grown and destructively harvested after one season of growth. Blocks of 2-year plants in each row were established in 2008 and harvested in 2009.

Plants for this study were grown from seed collected at the Glasgow, DE site in 2007, and seed dormancy was broken by removing a portion of the seedcoat with a scalpel. Seedlings were grown in a greenhouse mist-room, transplanted individually into pots, and maintained in a greenhouse as above. On 16 June 2008 when vines were approximately 1.2 m long and at the six leaf stage, holes were dug on the west side of each of the 70 plots, and lined with potting soil to limit contact with preemergence herbicides applied three days prior (13 June 2008; Mark VanGessel, personal communication). One plant was transplanted to each plot, and all plants were watered by hand for the first 14 d of the experiment. Mulch was placed at the base of plants to maintain soil moisture, and an irrigation system was installed with drip-tape (Ro-Drip 200 mm thickness, 30 cm spacing, 1.4 Kg per cm water flow) placed near the base of each plant for efficient watering as needed. Plants were inoculated on 27 June 2008 using Royal Peat garden combination humus seed inoculants (Becker Underwood; St. Joseph, MS), which contained a mixture of *Rhizobium leguminosarum*

*vicaeo*, *Rhizobium leguminosarum phaseoli*, and *Bradyrhizobium* spp., with 200 million of each species that were predicted to cross-inoculate kudzu (Whitt, 2000). A solution of 1050 ml distilled water and the inoculant mix was created, transported on ice to the farm, and 15 ml of solution was applied to the base of each plant. Plants were subsequently watered to help bacteria percolate through the soil.

Treatments included cutting 50 and 75% of each leaflet per leaf, clipping off the last 15 cm of 50 and 75% of the total number of stems, and a no-damage control. The block treatment (1- or 2-year plants) as well as individual treatments within a block were randomly assigned using the PLAN procedure in SAS (SAS Institute, 2008). Treatments were applied to all plants weekly for 8 wk beginning 7 July 2008. Leaf treatments were applied to all leaves during the first week, and to only newly emerged leaves thereafter. Above-ground biomass of 1-year plants was harvested on 1 September 2008. Vines were cut at the rootcrown, placed in individually marked plastic trash bags and stored at 2 °C. The lengths of all vines, including branches, were measured individually for each plant, and the number of nodes per length of vine was counted. These data were used to calculate average internode length. Above-ground biomass was separated into leaf and stem material, placed in paper bags and dried in a greenhouse for several weeks before weighing. On 22 October 2008, a 1 m diameter circle with the base of the plant at the center was dug to harvest roots of 1-year plants. Only live biomass with root diameters of > 2 mm was collected (root-hairs were not harvested), and several plants had no harvestable roots. These individuals were assigned a value of zero for root biomass, and were included as such in analysis. Collected roots were cleared of soil by washing with water, dried in paper bags and weighed. Two-year plants were left untouched at the end of 2008.

In 2009, preemergence herbicides metolachlor and imazethapyr were applied to the farm, and remaining weeds were removed by hand. For 2-year plants, only those in the 75% leaf cutting treatment and the no-damage control were studied in 2009, and plants received treatments for 4 wks starting 1 June. On 6 July 2009, all above- and below-ground biomass of control and 75% leaf cutting 2-year plants was harvested, including below-ground biomass from rooted stem nodes. A 1 m diameter circle was used for root harvest as above, and all plant biomass was put directly into paper bags and kept in a greenhouse for several months to dry before weighing.

Thirty-five plants grown from the same seed source and using the same propagation methods as in 2008 were transplanted to the farm on 8 June 2009 in plots where 1-year plants had been harvested. Plants were hand watered as needed. Treatments were applied once weekly for ten weeks starting 23 June 2009. Above- and below-ground biomass were harvested for 2009 1-year plants the week of 31 August. Plants were transported in large plastic trash bags, stored at 2 °C, vine lengths measured, nodes counted, and internode length calculated as above. Leaves and stems were not separated in 2009, but plants were placed in paper bags and dried for several weeks before weighing.

### **North Carolina Experiment**

In May 2007, a 67.5 by 72.5 m area on the Umstead Farm in Butner, NC was prepared by tilling, divided into six rows with twenty 1 by 10 m plots each, and plots were separated by 2 m alleys. Plants for this study were from stem cuttings of potted kudzu plants. Vines were cut 8 to 10 cm long, each with a stem node. Fully developed leaves were removed by clipping, and nodes were covered with all-purpose sand in 6 cm square pots and kept in a greenhouse room. On 17 September 2007, 120

plants were transplanted individually to the north end of the 1 by 10 m plots. The PLAN procedure (SAS Institute, 2008) was used to assign treatments to the 70 plants that survived to 2008. Starting at the north end of the plot, treatments were assigned from west to east in row one, from east to west in row two, etc. This method of assigning treatments minimized the distance between plants in the same block.

Damage treatments applied to plants in North Carolina were identical to those used in Delaware. Treatments were applied to plants weekly. An extended growing season in North Carolina permitted treatment application over a 16-wk period from 16 June to 29 September 2008. During this time, maintenance included mowing between plots and use of glyphosate for weed control. Above-ground biomass for all plants (1- and 2-year; separated into leaf and stem biomass), and below-ground biomass for 1-year plants was harvested on 20 October 2008. Roots were dug and soil was removed by hand. All plant material was dried in a curing barn at approximately 62.7 °C for 4 d before weighing.

In the fall of 2008, 35 stem nodes with roots were collected from a nearby kudzu patch, were potted and overwintered outdoors. In 2009, these plants were transplanted on 2 May to the same plots where 1-year plants had been harvested the previous October. Treatments were applied and the number of stem nodes was recorded for a 16-wk period from 1 June to 14 September. Plants that did not survive the transplant process were replaced during the first week. Above-ground biomass for all plants (1- and 2-year) was harvested the weeks of 21 and 28 September 2009, and plant material was separated into leaves and stems. On 5 October 2009, a backhoe (bucket size 50.8 cm<sup>3</sup>) was used to harvest below-ground biomass for all plants. Roots

were cleared of soil by hand and all plant biomass was dried in a curing barn for 4 d (62.7 °C) before weighing.

### **Statistical Analysis**

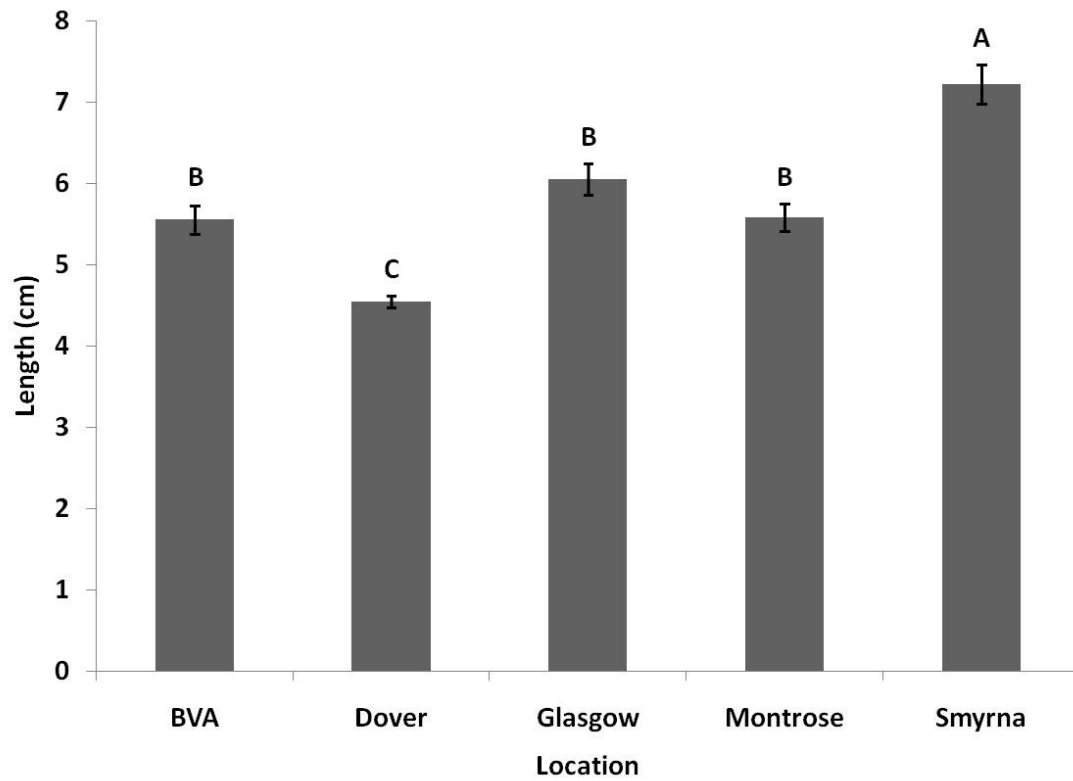
Statistical analyses were performed using SAS (SAS Institute, 2008). Pod length and the number of fully developed seeds per pod were compared among sites using analysis of variance (ANOVA, GLM procedure), and regression analysis (PROC REG) was used to determine if pod length could be used to predict seed number. For experiments on the Newark and Butner farms, response variables were examined with ANOVA by block. The Shapiro-Wilk test in the UNIVARIATE procedure was used to test the assumption of normality, and Levene's test in the GLM procedure was used to test for homoscedasticity of the data. The natural log transformation was used to adjust data that violated one or both of these assumptions. If transformed data also violated ANOVA assumptions, the nonparametric Kruskal-Wallis test of the NPAR1WAY WILCOXON procedure was used. These data were ranked and analyzed with ANOVA. Tukey's tests were used for mean separation. For the 2009 Delaware experiment, a Dunnett's test was used to compare the total combined vine lengths of individual treatments to the control at  $\alpha = 0.05$ .

## RESULTS

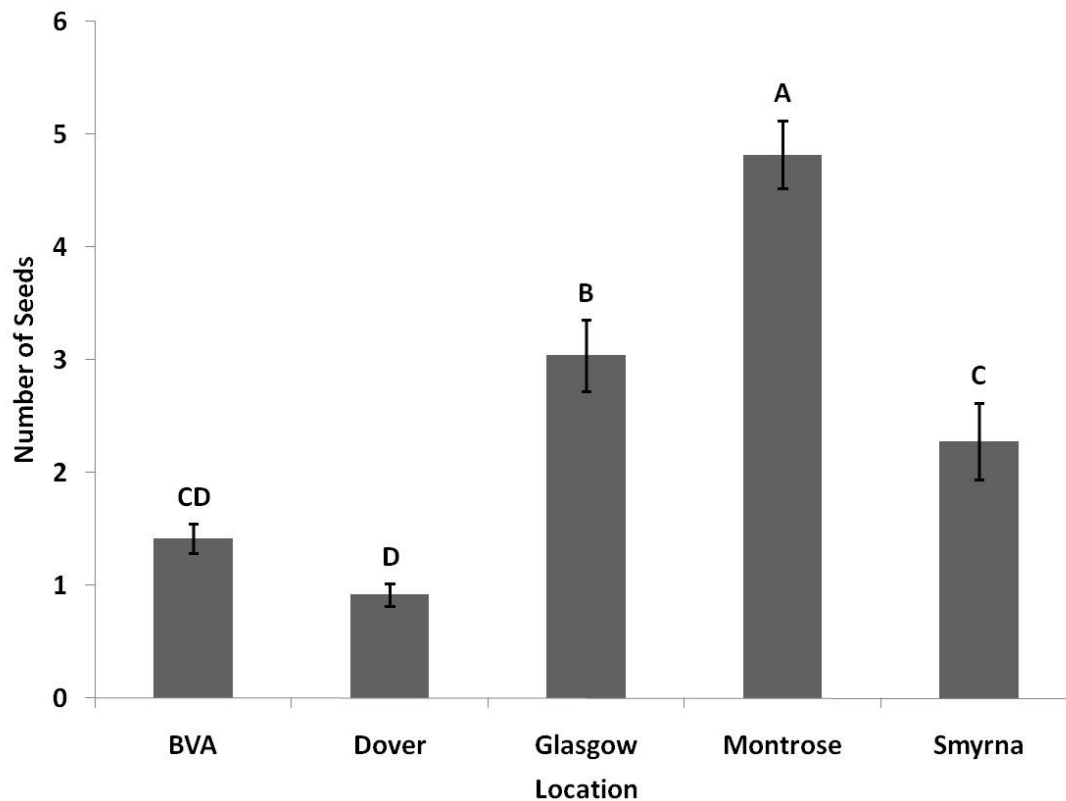
### Delaware Experiment

#### *Seed collection, emergence, and herbicide selection*

Despite a significant correlation ( $F_{1,249} = 8.35, P < 0.0001$ ), pod length was a poor predictor of the number of fully developed seeds per pod ( $r^2 = 0.2155$ ). Seedpods from Smyrna, DE were significantly longer than pods collected from all other sites (Figure 2.1), but pods from Glasgow, DE and Montrose, NY had significantly higher numbers of fully developed seed compared to the other sites (Figure 2.2).



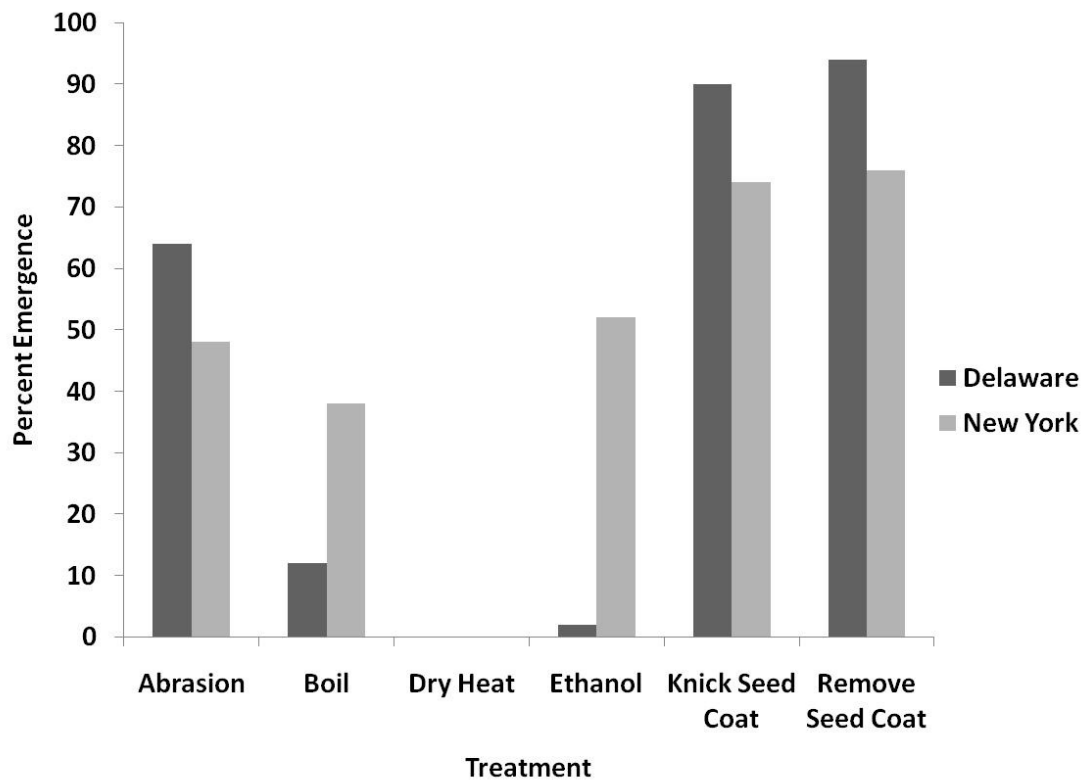
**Figure 2.1.** Length of seedpods collected from five kudzu patches. Means ( $\pm$  SEM) with the same letter are not significantly different ( $F_{4, 246} = 29.64$ ,  $P < 0.0001$  for ranked data,  $N = 50$  pods per site; Tukey's test used for mean separation).



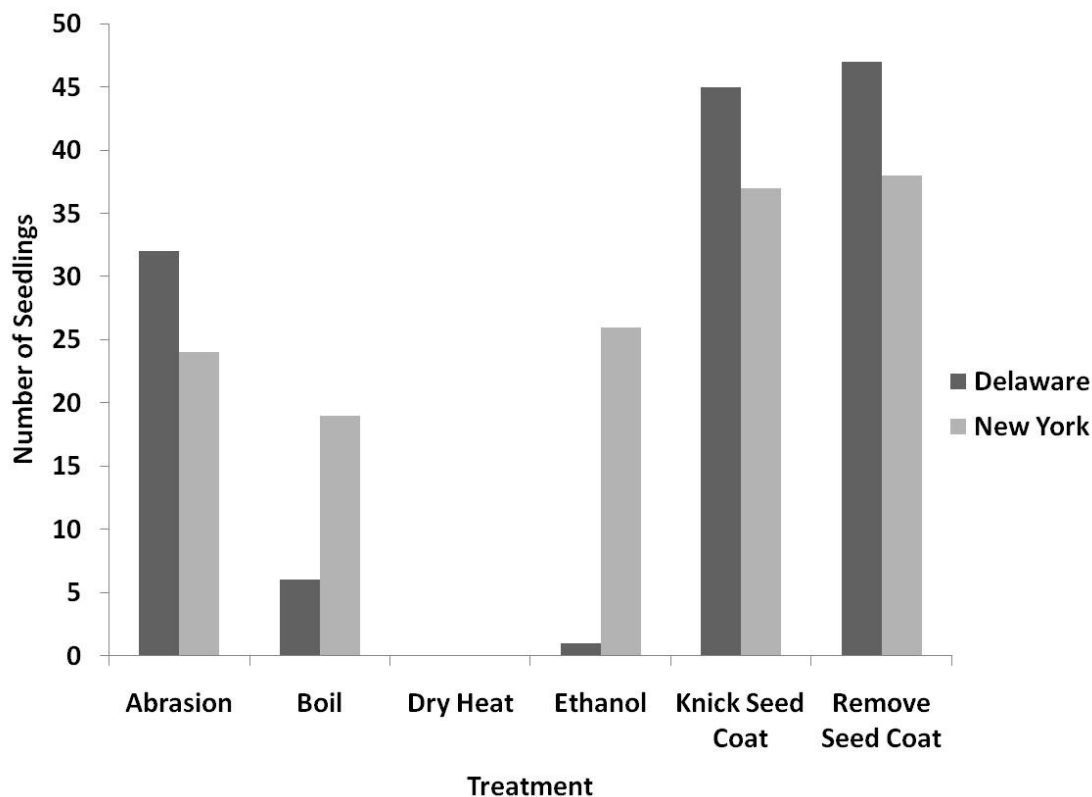
**Figure 2.2.** Number of fully developed seeds per pod from five kudzu patches. Means ( $\pm$  SEM) with the same letter are not significantly different ( $F_{4, 246} = 41.49$ ,  $P < 0.0001$  for ranked data,  $N = 50$  pods per site; Tukey's test used for mean separation).

Using a scalpel to nick, or remove a portion of the seedcoat resulted in the highest percent emergence of all treatments (Figure 2.3). For both techniques, emergence was higher for DE than NY seed. Sandpaper abrasion resulted in 64 and 48% emergence for DE and NY seed, and several seeds were crushed using this method. Emergence of seeds in the boiling and ethanol treatments was variable for the two seed sources. Dry heat was the least effective method for seed establishment, and no seeds emerged from either source with this technique (Figure 2.3). Nearly all seedlings from DE in which the seedcoat was knicked or cut survived to the two leaf

stage (Figure 2.4). Although percent emergence of seedlings in the boiling treatment was low, 83 and 90% of these seedlings from DE and NY survived to the two leaf stage (Figure 2.4).



**Figure 2.3.** Percent emergence under different treatments to break seedcoat dormancy ( $N = 50$  per treatment and location).



**Figure 2.4.** Seedling survival to the two leaf stage (18 d after planting). Absolute numbers presented to indicate overall success of each treatment in producing viable plants.

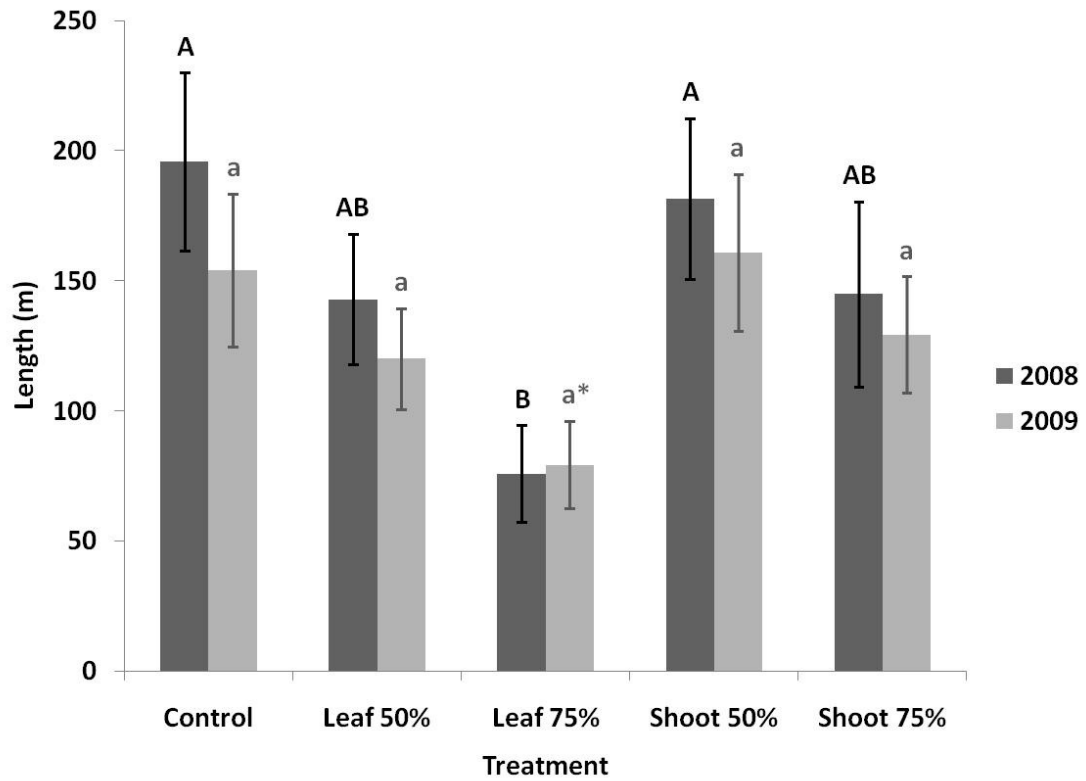
The herbicide fomesafen had negligible effects on kudzu growth. Minor speckling of leaves was observed and treatment plants appeared slightly stunted in their growth compared to the control. Imazethapyr also stunted growth slightly, and older leaves were chlorotic. However, new growth was green and plants seemed otherwise healthy. These two herbicides were considered safe for use if agricultural weeds became a problem in study plots. For the other herbicides studied, chlorimuron resulted in necrosis around leaf edges and near the midvein region of some leaves, yet new growth was noted. Halosulfuron-methyl burned expanding leaves, and plant

growth ceased after application. Cloransulam caused leaf bleaching of older leaves, and new leaves were deformed and curled. Mesotrione bleached new leaves killing plants, and atrazine was the most damaging herbicide causing leaf-burn as early as 3 d after application. No new growth was observed for any plant treated with atrazine.

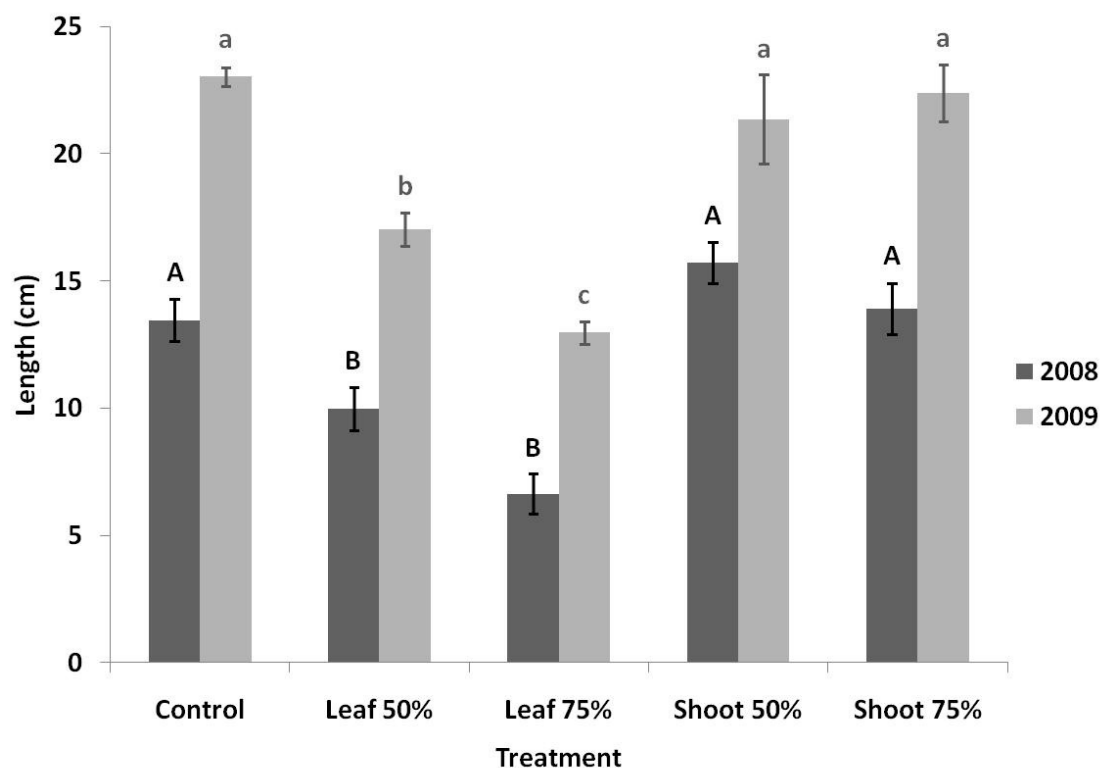
### ***Farm experiment***

For both one- and two-year plants in Delaware, there were no significant year by treatment interactions for any plant traits measured ( $P > 0.05$ , 2-way ANOVA). Preemergence herbicide applications effectively reduced weed problems in the plot in both years, and reapplication of these herbicides at the beginning of the second season did not appear to damage 2-year kudzu plants in 2009. For 1-year plants, there were no differences in the number of stem nodes by treatment at the end of the 2008 ( $F_{10, 23} = 0.63$ ,  $P = 0.6454$ ) or 2009 seasons ( $F_{10, 24} = 0.69$ ,  $P = 0.6038$ ).

Seventy-five percent leaf cutting significantly reduced total vine length in 2008 and 2009 compared to the control (Figure 2.5). Average internode lengths were shorter for all plants in 2008 than in 2009 ( $F_{9, 60} = 122.82$ ,  $P < 0.0001$ ), and shoot clipping treatments were not significantly different from the control in either year (Figure 2.6). However, 50 and 75% leaf cutting significantly reduced internode length in both years (Figure 2.6).

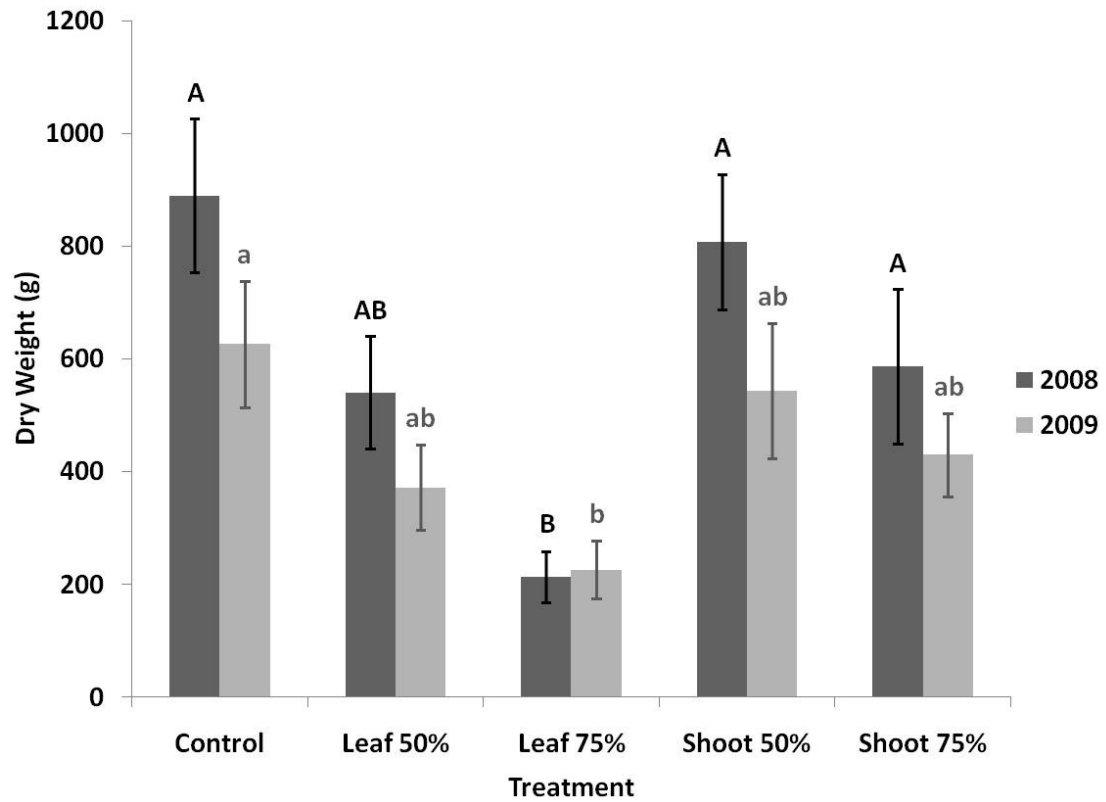


**Figure 2.5.** Total vine length for 1-year plants from Delaware in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{10,23} = 4.24$ ,  $P = 0.0103$ ,  $N = 6$  for the Shoot 75% treatment, 7 for all other treatments; 2009:  $F_{10,24} = 2.62$ ,  $P = 0.0603$ ,  $N = 7$  for all treatments; Tukey's test used for mean separation). \* indicates a significant difference from the control ( $\alpha = 0.05$ , Dunnett's Test).



**Figure 2.6.** Average internode length for 1-year plants from Delaware in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{10, 23} = 20.03$ ,  $P < 0.0001$ ,  $N = 6$  for the Shoot 75% treatment, 7 for all other treatments; 2009:  $F_{10, 24} = 22.97$ ,  $P < 0.0001$ ,  $N = 7$  for all treatments; Tukey’s test used for mean separation).

Compared to the control, above-ground biomass was significantly reduced for 1-year plants in the 75% leaf cutting treatment in both 2008 and 2009 (Figure 2.7), with reductions of 76 and 64%, respectively. Leaf dry weight in 2008 accounted for approximately half or more of the total above-ground biomass within each treatment despite losses to plant material by cutting or clipping treatments (Table 2.1).

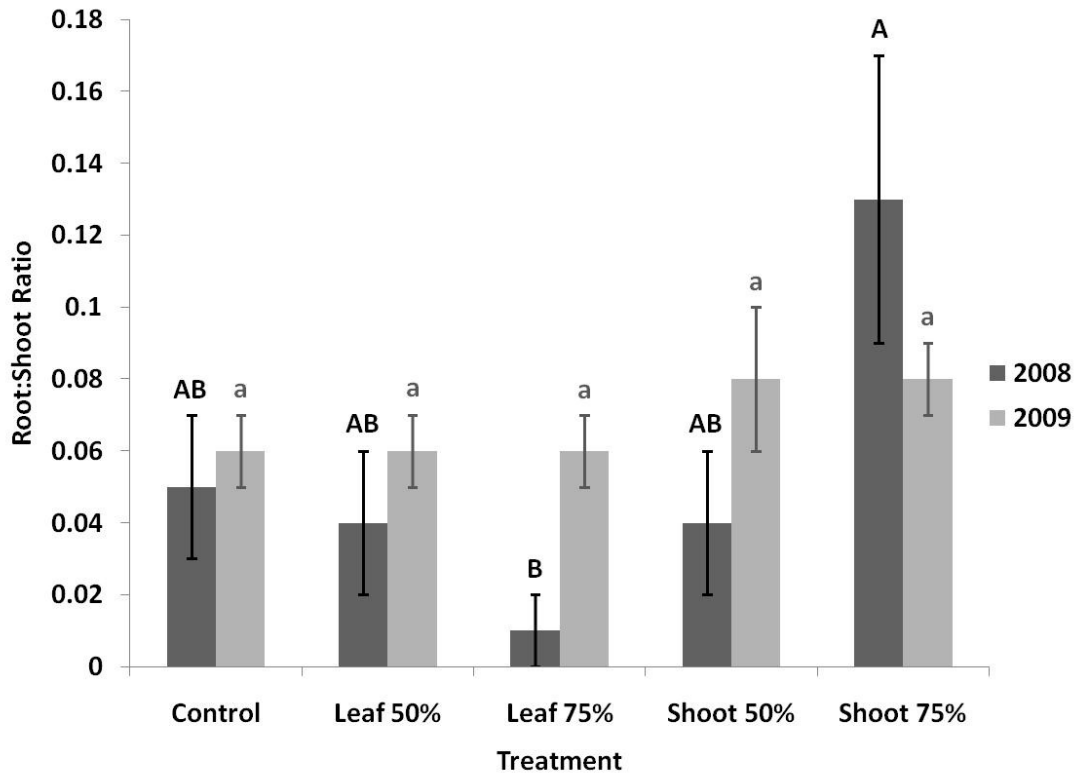


**Figure 2.7.** Above-ground biomass for 1-year plants from Delaware in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{10, 23} = 9.75$ ,  $P < 0.0001$ ,  $N = 6$  for the Shoot 75% treatment, 7 for all other treatments; 2009:  $F_{10, 24} = 3.85$ ,  $P = 0.0149$ ,  $N = 7$  for all treatments; Tukey's test used for mean separation).

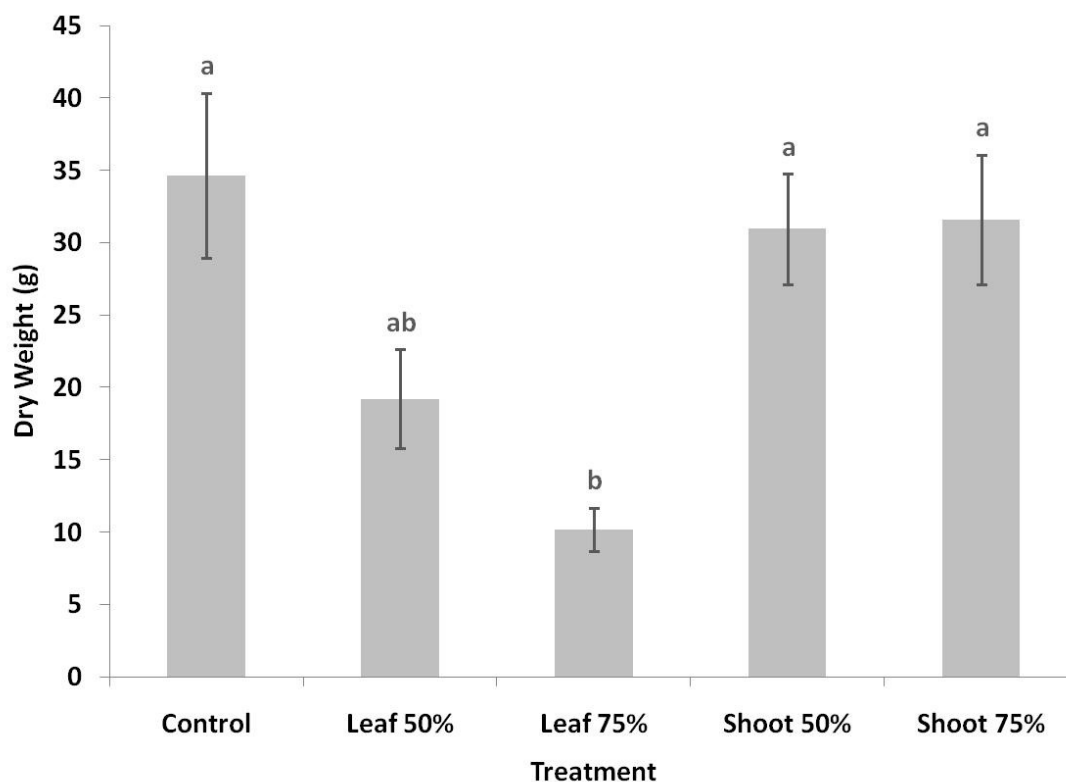
**Table 2.1.** Leaf and stem biomass for 1-year plants from Delaware in 2008 ( $N = 6$  for Shoot 75%, 7 for all other treatments) and North Carolina in 2008 and 2009 ( $N = 7$  per treatment in both years). Data are means  $\pm$  SEM.

|                     | <b>Treatment</b>   |                    |                    |                    |                    |
|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                     | <b>Control</b>     | <b>Leaf 50%</b>    | <b>Leaf 75%</b>    | <b>Shoot 50%</b>   | <b>Shoot 75%</b>   |
| Delaware 2008       |                    |                    |                    |                    |                    |
| Leaf Biomass        | 425.94 $\pm$ 60.46 | 254.86 $\pm$ 43.91 | 109.49 $\pm$ 21.94 | 400.49 $\pm$ 53.90 | 303.22 $\pm$ 66.48 |
| Stem Biomass        | 463.84 $\pm$ 76.83 | 285.16 $\pm$ 56.04 | 103.79 $\pm$ 23.66 | 407.01 $\pm$ 67.04 | 283.07 $\pm$ 66.48 |
| % Leaf biomass      | 48%                | 47%                | 51%                | 50%                | 52%                |
| North Carolina 2008 |                    |                    |                    |                    |                    |
| Leaf Biomass        | 166.24 $\pm$ 22.88 | 65.30 $\pm$ 18.36  | 37.12 $\pm$ 11.69  | 41.78 $\pm$ 14.26  | 57.21 $\pm$ 15.44  |
| Stem Biomass        | 159.47 $\pm$ 33.65 | 45.23 $\pm$ 15.20  | 20.61 $\pm$ 6.90   | 19.35 $\pm$ 9.56   | 34.73 $\pm$ 13.51  |
| % Leaf biomass      | 51%                | 59%                | 64%                | 68%                | 62%                |
| North Carolina 2009 |                    |                    |                    |                    |                    |
| Leaf Biomass        | 288.80 $\pm$ 33.89 | 75.80 $\pm$ 16.45  | 45.91 $\pm$ 10.41  | 131.56 $\pm$ 36.07 | 85.09 $\pm$ 31.64  |
| Stem Biomass        | 239.16 $\pm$ 28.73 | 53.03 $\pm$ 15.47  | 27.39 $\pm$ 6.32   | 116.98 $\pm$ 35.31 | 53.02 $\pm$ 23.04  |
| % Leaf biomass      | 55%                | 59%                | 63%                | 53%                | 62%                |

For 1-year plants that had harvestable, live root biomass at the end of 2008, there were no differences among treatments ( $F_{4, 29} = 2.20$ ,  $P = 0.0940$  for ranked data;  $N = 5$  for Control, 75% Shoot Clip; 3 for 50% Leaf Cut, 50% Shoot Clip; 1 for 75% Leaf Cut). However, the root:shoot ratio for plants in the 75% leaf cutting treatment was significantly reduced compared to 75% shoot clipping in 2008 (Figure 2.8). In 2009 root biomass was significantly reduced for plants in the 75% leaf cutting treatment compared to the control and shoot clipping treatments (Figure 2.9), but there was no difference in the root:shoot ratio (Figure 2.8).



**Figure 2.8.** Root:shoot ratio for 1-year plants from Delaware in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{4, 29} = 3.30$ ,  $P = 0.0241$  for ranked data,  $N = 6$  for the Shoot 75% treatment, 7 for all other treatments; 2009:  $F_{10, 24} = 1.81$ ,  $P = 0.1599$  for natural log transformed data,  $N = 7$  per treatment; Tukey's test used for mean separation).



**Figure 2.9.** Root biomass for 1-year plants from Delaware in 2009. Means ( $\pm$  SEM) with the same letter are not significantly different ( $F_{10, 24} = 6.84$ ,  $P = 0.0008$ ,  $N = 7$  for all treatments; Tukey's test used for mean separation).

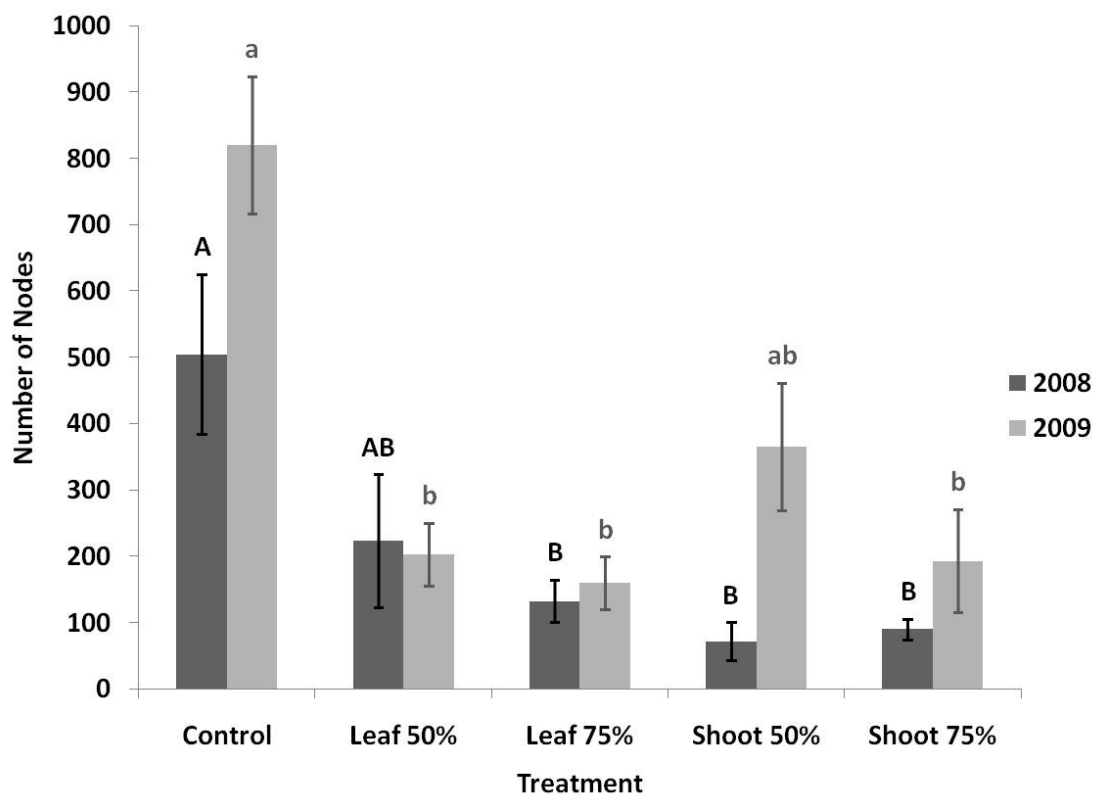
Survival of 2-year plants to 2009 was 100%, and plants on the farm began growing the week of 27 April. No differences were detected for above-ground biomass, root biomass, or the root:shoot ratio of harvested 2-year plants (Table 2.2).

**Table 2.2** Biomass (mean  $\pm$  SEM) of 2-year plants from Delaware harvested in 2009.

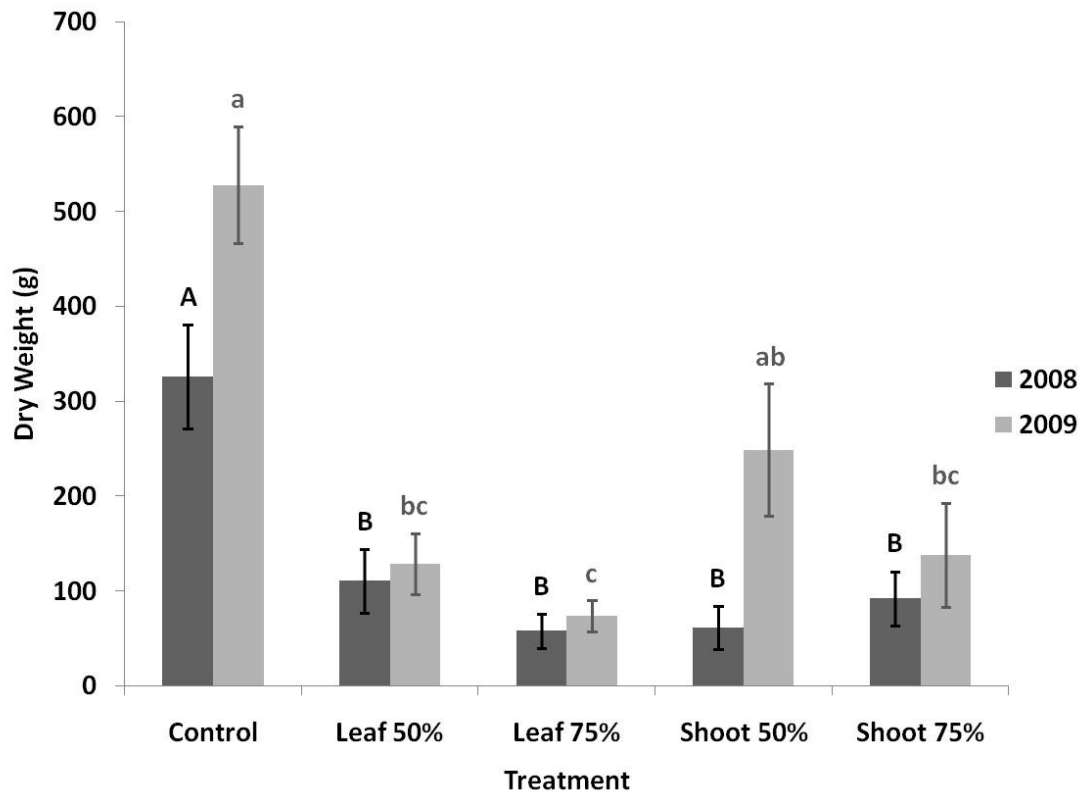
|             | Root Biomass (g)   | Above-ground Biomass (g) | Root:Shoot Ratio |
|-------------|--------------------|--------------------------|------------------|
| Control     | 303.01 $\pm$ 68.53 | 2914.37 $\pm$ 790.17     | 0.12 $\pm$ 0.01  |
| Leaf 75%    | 196.7 $\pm$ 47.08  | 1464.44 $\pm$ 315.62     | 0.13 $\pm$ 0.01  |
| $F_{2, 11}$ | 1.50               | 2.51                     | 1.38             |
| $P$         | 0.2465             | 0.1415                   | 0.2653           |

## North Carolina Experiment

Above-ground biomass of 1-year plants in North Carolina was lower than Delaware, with control plants in NC producing an average of  $325.7 \pm 55.0$  and  $528.0 \pm 61.7$  g, versus  $889.8 \pm 135.8$  and  $626.17 \pm 112.5$  g for DE in 2008 and 2009, respectively. For both one- and two-year plants in NC, there were no significant year by treatment interactions for any plant trait measured ( $P > 0.05$ , 2-way ANOVA). Seventy-five percent leaf cutting and 75% shoot clipping significantly reduced the number of stem nodes in both years (Figure 2.10). Above-ground biomass production was reduced by all damage treatments compared to the control, except for 50% shoot clipping in 2009 (Figure 2.11). In 2008, above-ground biomass production was reduced by 66% or greater for all treatments, with the highest reduction of 82% observed for plants in the 75% leaf cutting treatment (Figure 2.11). Percent reduction in above-ground biomass of 1-year plants compared to the control in 2009 ranged from 76 to 86%. Despite these reductions in overall biomass, leaf biomass accounted for more than half of the total plant biomass for all 1-year plants in 2008 and 2009 (Table 2.1).



**Figure 2.10.** Average number of stem nodes for 1-year plants from North Carolina in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{10,24} = 7.26$ ,  $P = 0.0006$ ; 2009:  $F_{10,24} = 5.54$ ,  $P = 0.0026$ ; for both years data were natural log transformed,  $N = 7$  per treatment; Tukey's test used for mean separation).

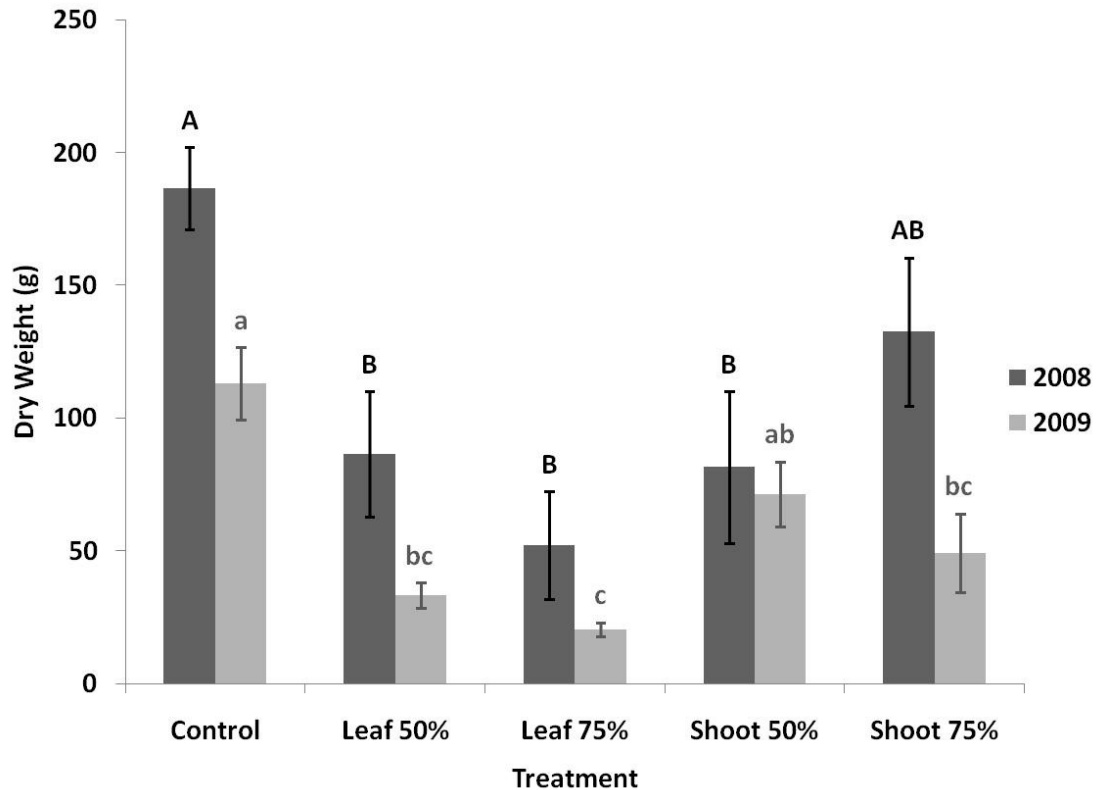


**Figure 2.11.** Above-ground biomass for 1-year plants from North Carolina in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{4,30} = 6.59$ ,  $P = 0.0006$ ; 2009:  $F_{4,30} = 8.98$ ,  $P < 0.0001$ ; for both years ranked data were analyzed with ANOVA,  $N = 7$  per treatment; Tukey's test used for mean separation).

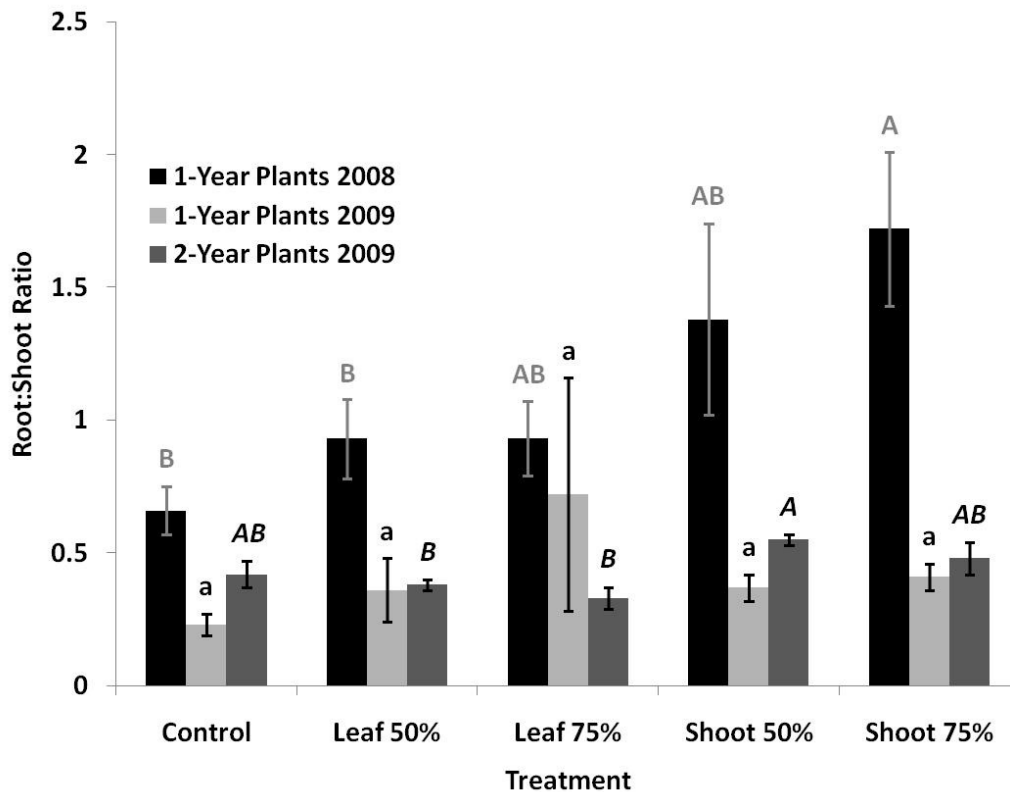
Root biomass in NC was higher than in DE, with control plants in NC producing an average of  $186.4 \pm 15.52$  and  $113.0 \pm 13.58$  g, versus  $56.1 \pm 17.39$  and  $34.7 \pm 5.69$  g for DE in 2008 and 2009, respectively. In NC, 50 and 75% leaf cutting significantly reduced root biomass of 1-year plants in 2008 and 2009 (Figure 2.12). The highest percent reduction in root biomass compared to the control in both years was for 75% leaf cutting, which decreased biomass by 72% in 2008 and 81% in 2009 (Figure 2.12). The root:shoot ratio in 2008 was highest for plants in the 75% shoot

clipping treatment, and lowest for the control and 50% leaf cutting (Figure 2.13).

There were no differences in the root:shoot ratio in 2009 (Figure 2.13).



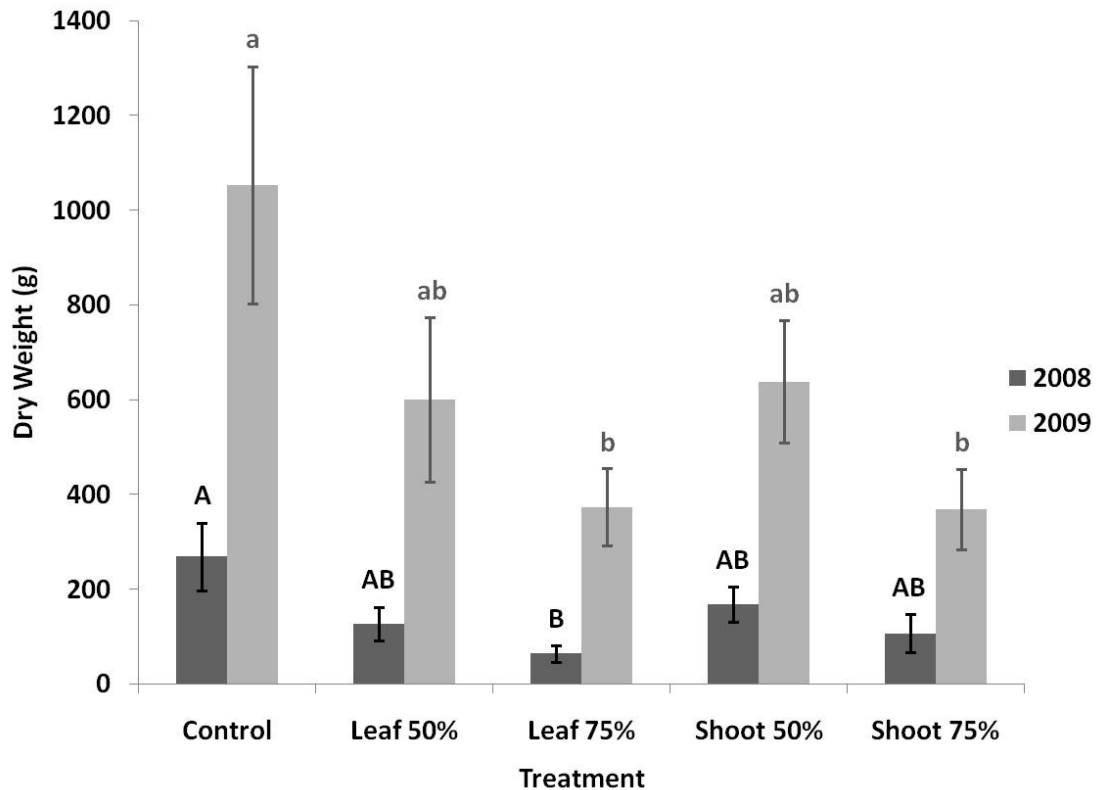
**Figure 2.12.** Root biomass for 1-year plants from North Carolina in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{10, 24} = 4.99$ ,  $P = 0.0045$ ; 2009:  $F_{10, 24} = 10.55$ ,  $P < 0.0001$  for natural log transformed data;  $N = 7$  per treatment in both years; Tukey's test used for mean separation).



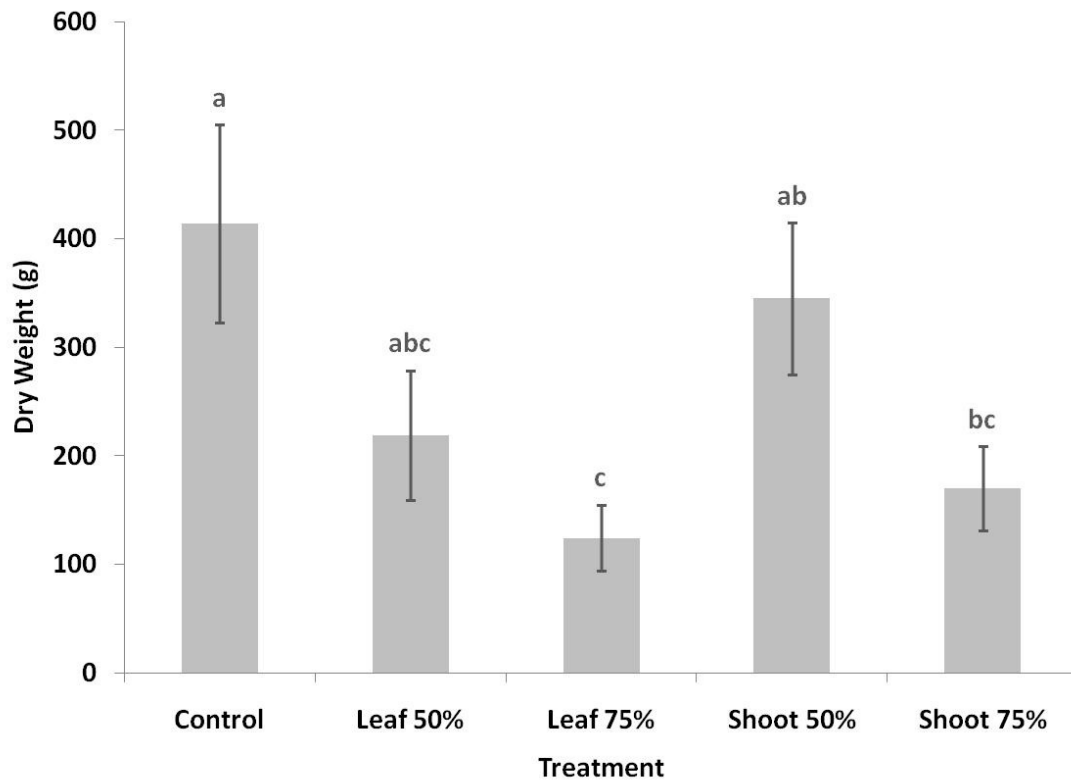
**Figure 2.13.** Root:shoot ratio for plants from North Carolina in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (1-Year 2008:  $F_{4,30} = 5.49$ ,  $P = 0.0019$  for ranked data analyzed with ANOVA; 1-Year 2009:  $F_{4,30} = 2.15$ ,  $P = 0.0992$  for ranked data analyzed with ANOVA; 2-Year 2009:  $F_{10,24} = 4.53$ ,  $P = 0.0072$ ,  $N = 7$  per treatment; Tukey's test used for mean separation).

For 2-year plants at the end of the season, the number of stem nodes ( $F_{9,60} = 25.09$ ,  $P < 0.0001$ ) and above-ground biomass ( $F_{9,60} = 39.83$ ,  $P < 0.0001$ ; Figure 2.14) were significantly higher in 2009 compared to 2008. However, there were no differences in the number of stem nodes by treatment for 2-year plants in 2008 ( $F_{10,24} = 2.50$ ,  $P = 0.0693$  for natural log transformed data) or 2009 ( $F_{10,24} = 2.95$ ,  $P = 0.0407$  for natural log transformed data; no differences detected with Tukey's test). In both years, 75% leaf cutting significantly reduced above-ground biomass compared to

the control, and 75% shoot clipping produced less biomass than the control in 2009 only (Figure 2.14). Root biomass for 2-year plants was significantly reduced by 75% leaf cutting and 75% shoot clipping compared to the control (Figure 2.15). The root:shoot ratio for 2-year plants in the leaf cutting treatments were not significantly different from the control, but were all significantly reduced compared to 50% shoot clipping (Figure 2.13).



**Figure 2.14.** Above-ground biomass for 2-year plants from North Carolina in 2008 and 2009. Within a year, means ( $\pm$  SEM) with the same letter are not significantly different (2008:  $F_{10,24} = 3.74$ ,  $P = 0.0167$ ; 2009:  $F_{10,24} = 5.04$ ,  $P = 0.0043$ ; for both years data were natural log transformed,  $N = 7$  per treatment; Tukey's test used for mean separation).



**Figure 2.15.** Root biomass for 2-year plants from North Carolina in 2009. Means ( $\pm$  SEM) with the same letter are not significantly different ( $F_{10,24} = 7.26$ ,  $P = 0.0006$  for natural log transformed data,  $N = 7$  per treatment; Tukey's test used for mean separation).

## DISCUSSION

The goal of the current study was to better understand the growth response of newly established kudzu plants to different types and levels of simulated herbivore damage near the center (North Carolina) and edge (Delaware) of the plant's invasive range. We accomplished this by conducting a manipulative experiment in an agricultural field, where plants had access to full light conditions and were not subject to competition from surrounding plants. High levels of leaf damage (75% removal) reduced above- and below-ground biomass compared to an undamaged control for 1-

and 2-year old kudzu plants in North Carolina, and for 1-year old plants in Delaware, and also reduced total vine length and average internode length when measured in Delaware.

A common concern for efficacy trials that investigate individual plant response is that results may not translate to population-level effects for invasive species (McClay and Balciunas, 2005). Regarding kudzu, previous experiments in large research plots (15.25 by 30.5 m) showed that total biomass production was highest for plants cut multiple times in the first of a two-year study (Terrill et al., 2003). In the second year, however, the opposite result was found such that plots cut three times the first year yielded the least amount of plant material in the second year (Terrill et al., 2003). This result supports the hypothesis that kudzu compensates for above-ground biomass removal at the expense of below-ground resources, and suggests that foliar damage might provide control of kudzu even at the population level. This is in contrast to predictions from other invasive weed systems, where plants with large root systems, like kudzu, are thought to require root-feeding insects that directly attack and deplete these structures.

For particular weed species, simulated herbivory research has identified the root-feeding guild as the most harmful to individual plants (Broughton, 2003), and released root-feeding biological control agents show great potential to impact weed populations (Blossey and Hunt-Joshi, 2003). However, the results of this and other studies demonstrate that indirect damage to above-ground structures can be equally devastating to plants by affecting root resources (Davies et al., 2005; Kleinjan et al., 2004; Raghu et al., 2006). For example, removing above-ground foliage of *Ulex europaeus* L. (Fabaceae) by clipping with scissors reduced root dry weights by 70%

compared to an undamaged control (Davies et al., 2005), while damage to leaf and stem mesophyll cells by a leafhopper, *Zygina* sp. (Hemiptera: Cicadellidae), reduced tuber production rates for the invasive bridal creeper *Asparagus asparagoides* (L.) in Australia (Kleinjan et al., 2004). In the current study, we observed that leaf damage in general, and 75% removal in particular, significantly reduced root biomass production. In Delaware and North Carolina, root:shoot ratios for 1- and 2-year plants in the 75% leaf cutting treatment were close to, or below a value of one, suggesting a higher allocation of resources to above-ground plant parts compared to roots. With roughly equal stem and leaf biomass for all damage treatments, even in cases where 75% of leaf biomass was removed, these results indicate that kudzu compensates for loss of photosynthetic surfaces at the expense of below-ground resources. Because the plant relies on stored root carbohydrates to produce new growth in the spring (Wechsler, 1977) and to enhance drought tolerance (Forseth and Innis, 2004), our results support previous findings that depleting root stores via leaf damage over several years can decrease plant survival (Miller and Edwards, 1983).

Plant mortality did not result from simulated herbivory treatments in this or other studies (Schat and Blossey, 2005; Wirf, 2006). However, we observed that 75% leaf cutting was sufficient to decrease above-ground biomass, total vine length and average internode length of kudzu in both years of the study in Delaware. Shorter internodes are a symptom of plant stress, and can result from nutrient deficiencies, water limitation (Taiz and Zeiger, 2006) and herbivory (Lamp et al., 2007). For kudzu in particular, shorter internodes might indicate reduced vine elongation rates. Reductions in internodes, total vine lengths, and biomass production above- and below-ground should combine to limit the spread of kudzu by vegetative propagation,

and might alter the competitive ability of the plant in natural settings. This is because removal of leaf tissue not only disrupts a plant's photosynthetic apparatus, but can produce additional effects such as increased light penetration below the weed canopy (Peterson et al., 1992; Zangerl et al., 2002), providing an opportunity for other plants to compete with kudzu. For example, leaf-feeding by *Galerucella californiensis* L., a biological control agent released against purple loosestrife (*Lythrum salicaria* L.), increased light penetration through a previously dense canopy of this weed (Hunt-Joshi et al., 2004). Native plants benefitted from this herbivory by producing more above-ground biomass, and were subsequently better able to compete with *L. salicaria*. By integrating control methods such as revegetation techniques, the use of herbicides, grazing by mammals, and controlled burns (Davies et al., 2005; Lym, 2005), high levels of leaf damage could reduce the competitive superiority of kudzu in natural areas: a primary goal in biological control.

In our study, we observed similar relationships among treatments within a year, but noted differences in overall plant production between years and sites. For instance, above-ground biomass production for 1-year plants was higher in 2008 than 2009 in Delaware, while the opposite relationship was found in North Carolina. One explanation for these differences is variation in resource availability. Compared to 2008, early spring 2009 in Delaware was cooler, with an additional 10 cm of rain during the growing season. While lower temperatures and presumably reduced light reception would have slowed the growth of plants in 2009, elongation rates of vines are often higher with enhanced water availability (Taiz and Zeiger, 2006), accounting for longer internodes in 2009 than in 2008.

Lower above-ground biomass production for plants in North Carolina compared to Delaware was contrary to our expectations, but might also be resource based. At the beginning of the 2008 season, plants in Delaware were provided with rhizobium known to cross-inoculate kudzu, and root nodules were observed during harvest of below-ground plant parts in both years (personal observation). Enhanced nitrogen availability for Delaware plants might have overwhelmed differences between the two sites, including a longer growing season in North Carolina. Additionally, temperature differences between the center and edge of kudzu's invasive range could have reduced biomass production in North Carolina. The optimal temperature range for kudzu photosynthesis is between 18 and 33 °C, while temperatures above 33 °C are thought to be deleterious for the plant (Wechsler, 1977). Although kudzu can alter leaf orientations to reduce the surface temperature of leaves by up to 6 °C and prevent excessive water loss (Forseth and Teramura, 1986), individual plants grown in an open field might have experienced elevated temperature and water stress that limited plant productivity. If true, this might explain why an additional 15 cm of rain in North Carolina in 2009 marginally increased plant productivity for all treatments. To explicitly test differences in plant growth between North Carolina and Delaware, and to elucidate the factors causing the observed differences, it would be necessary to replicate this study at multiple sites and document differences in resource availability by region.

In Delaware we observed no differences in the numbers of stem nodes per plant by treatment, but in North Carolina there were significant reductions for all treatments compared to the control. Although stem nodes have been used as a reproductive measure in other systems (Schooler et al., 2006), our results here and

those reported previously (Chapter 1) indicate that this metric might not be informative for kudzu. Therefore, we recommend the use of measurements that incorporate stem node numbers, such as average internode length, or plant level metrics such as biomass, over stem node counts when considering the response of kudzu to herbivory.

In China, a total of 116 insect species were collected from kudzu in six feeding guilds (Sun et al., 2006). However, detailed life history information for individual species, specifically the position and extent of damage to the plant, is not known. Therefore, the current study investigated plant response to simulated insect feeding damage in guilds of potentially specific biological control agents (Sun et al., 2006). High levels of 50 and 75% removal of shoots and leaves were selected to elicit and accentuate plant response to herbivore stress, as insects imported for biological control can experience outbreak population dynamics (Keane and Crawley, 2002) and completely defoliate target plants (e.g., Hudgeons et al., 2007). The results of our study suggest that future efforts to identify host specific biological control agents for kudzu should focus on insects in the leaf-feeding guild. While one defoliating insect has been rejected due to the potential for non-target effects (Frye et al., 2007), Sun et al. (2006) have identified two potentially host specific foliage-feeding species and 14 defoliators with unknown host ranges in China. Therefore, because insects that produce high levels of leaf damage have the potential to reduce total plant biomass, total vine length, internode length, and alter resource allocation, host range evaluation of these insects should be a priority.

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## Chapter 3

# PLANT ARCHITECTURE AND BIOMASS RESPONSE OF KUDZU (*PUERARIA MONTANA* VAR. *LOBATA*) TO SIMULATED AND ACTUAL INSECT HERBIVORY

## INTRODUCTION

In 1876 kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & S. M. Almeida ex Sanjappa & Predeep (Suddath) (Fabaceae), was intentionally introduced to the United States from Asia (Mitich, 2000). Only after extensive plantings were made throughout the southeastern United States was it discovered that the rapid growth rate and climbing habit of this plant make it a nuisance (Blackwell, 1973). Since 1999, kudzu has been targeted for biological control (Britton et al., 2002), but no natural enemies have been released to date (Frye et al., 2007).

The focus of recent work in the biological control program targeting kudzu is to document plant response to different types and levels of simulated herbivore damage for plants in established patches (Chapter 1) and for newly established plants under ideal growing conditions in agricultural settings (Chapter 2). With over 100 insects collected on kudzu in China (Sun et al., 2006), the goal of these studies is to identify the insect feeding guild that will successfully reduce plant growth and reproduction in the introduced range. An example of how this technique can be used in practice is illustrated by the biological control program targeting cat's claw creeper,

*Macfadyena unguis-cati* (L.) Gentry (Bignoniaceae). In several countries, *M. unguis-cati* is an invasive weed that causes ecological damage in riparian communities. It is a woody, climbing vine that reproduces sexually by seed and vegetatively from adventitious roots on prostrate stems. Roots produced by the plant will become tuberous with age (Raghu et al., 2006, and references therein), making *M. unguis-cati* similar in growth form and habit to kudzu. As part of the biological control program to reduce the damage caused by this plant, Raghu and Dhileepan (2005) conducted simulated herbivory studies examining different damage treatments individually, and in combination, at multiple frequencies. Results demonstrated that at least two severe defoliation events reduce the plant's climbing habit and allocation to tubers (Raghu et al., 2006), while direct damage to tubers had a stimulatory effect on plant biomass production. Based on these findings, the guild of root-feeding herbivores was ranked lower than defoliators for their impact on *M. unguis-cati* (Raghu and Dhileepan, 2005; Raghu et al., 2006). This information was used to narrow the search for biological control agents from the plant's native range and shifted the focus to leaf-damaging insects. A defoliating chrysomelid beetle has been released (*Charidotis auroguttata* (Boheman)); in addition, two lacebugs (Tingidae) that decrease chlorophyll content of leaves are host specific and potentially damaging to the plant, but have not yet been released (Williams et al., 2008). Postrelease evaluation of insect impacts on *M. unguis-cati* is needed to determine if prerelease simulated herbivory studies accurately predicted impact of released biological control agents.

An important consideration for studies like the one conducted by Raghu et al. (2006) is how impacts on individual plants predicted by simulated herbivory will translate to natural situations (Hjältén, 2004). In general, simulated herbivory studies

are valued for their ability to (1) control the exact amount and timing of imposed damage (Baldwin, 1990), and (2) standardize biotic and abiotic conditions that might vary naturally (Hjältén, 2004). Furthermore, these studies allow researchers to obtain important ecological information about plant response to damage prior to importation and quarantine testing of potential biological control agents (Schooler et al., 2006). Because weeds can undergo postintroduction genetic changes (Müller-Schärer et al., 2004) that might alter plant response compared to the country of origin, tests of plant physiological tolerance and resistance to herbivory should be conducted in the introduced range. Despite the benefits of these studies, the use of simulated herbivory has been criticized for an inability to elicit physiological (Schooler et al., 2006) and chemical responses (Baldwin, 1988) identical to those produced by actual herbivory. Lehtilä and Boalt (2004) reviewed results from a number of experiments comparing actual and simulated herbivory, and found that plant response differed in 75% of studies examining plant phytochemistry. However, physiological tolerance responses such as growth and reproduction were often similar for simulated and actual herbivory treatments (Lehtilä and Boalt, 2004).

Recent studies have compared plant response to simulated and actual herbivory for released or potential biological control agents (Schat and Blossey, 2005; Schooler et al., 2006; Wirf, 2006). The timing, amount and frequency of damage applied to plants in these studies were determined from native range information, yet results constitute a spectrum of plant response to herbivory. Specifically, Schat and Blossey (2005) were unable to accurately mimic insect damage and obtained different results for the two types of herbivory for all measured variables, while Schooler et al. (2006) found that biomass was the same between simulated and actual herbivory

treatments, but stem length and the number of stem nodes were different. Finally, Wirf (2006) found that plants subject to simulated and actual herbivore damage produced the same results for all measured variables. Regardless of differences in plant response to actual and simulated herbivory, researchers were able to obtain important demographic data about each invasive species from control plants, information that is often lacking in the introduced range (Raghu and Dhileepan, 2005).

In previous studies with kudzu, it has been shown that high levels of leaf removal were sufficient to reduce several growth parameters of damaged plants, including internode length, total vine length, above-ground biomass, root biomass, and the root:shoot ratio (Chapter 2). However, little is known about how the plant will respond to actual insect herbivory if future leaf-feeding biological control agents prove sufficiently host-specific for release. Therefore, the current study was undertaken to supplement the findings of simulated herbivory experiments, and to fully characterize plant growth responses to simulated and actual herbivory for biomass production and plant architecture. Simulated damage, 75% leaf removal by cutting, was compared to actual damage imposed by larvae of the silver-spotted skipper *Epargyreus clarus* Cramer (Hesperiidae), a native herbivore that feeds, and can complete its development on kudzu.

## **MATERIALS AND METHODS**

Kudzu plants were grown from seed collected in Glasgow, DE in 2007. A portion of the seedcoat was removed using a scalpel to break coat-imposed dormancy (Susko et al., 2001). Seeds were sown in flats of redi-earth (Sun Gro Horticulture Canada Ltd.; Vancouver, British Columbia) on 26 March 2009, and placed in a mist

room that waters plants for 1 min six times per day. Seedlings were transplanted to 15-cm diameter round pots with Pro-mix (Premier Horticulture; Red Hill, PA) on 17 April, watered twice daily by hand, fertilized once per week with a 200 ppm nitrogen solution, and grown in a greenhouse at 18 to 22 °C. On 15 June 2009, plants reached the six leaf stage (approximately 1.2 m vine length) and were transplanted to the Newark Farm.

A 26 by 19 m farm field on the Newark Farm was tilled and treated with 1.5 L per hectare of *S*-metolachlor (Dual Magnum, Syngenta Crop Protection, Inc.; Greensboro, NC) and 0.15 L per hectare of imazethapyr (Pursuit, BASF Corporation; Florham Park, NJ) as preemergence herbicides to reduce competition from agricultural weeds (Mark VanGessel, University of Delaware, personal communication). The tilled area was divided into eight 1 m rows with 2 m alleys, and each row contained three 1 by 5 m plots. Plots within a row were separated by 1 m alleys. Holes were dug on the west side of each plot, lined with compost, and individual kudzu plants were transplanted and watered as needed. Treatments were applied in a randomized complete block design, with rows serving as blocks, and included: simulated herbivore damage as 75% leaf cutting, actual damage imposed by *E. clarus* larval feeding, and a no-damage control. Seventy-five percent leaf cutting was achieved by removing the right half of each leaflet per leaf, and then cutting the remaining leaflet in half so that only the lower left quarter of each leaflet remained intact. This damage was applied to all leaves on 3 August 2009, and to new, fully expanded leaves one per week thereafter until 31 August 2009.

For actual damage, larvae of the silver-spotted skipper were collected from kudzu patches in Glasgow, DE and Elkton, MD once per week from 3 August to

31 August 2009. Larvae of this native skipper feed on legumes including *Robinia pseudoacacia* L. (black locust), and kudzu (Weiss et al., 2003, and references therein). The larvae make characteristic tent-like shelters on leaf margins that vary predictably with insect age, and are created when caterpillars make two incisions from the leaf edge, then use silk to hold down the resulting flap (Weiss et al., 2003). With the exception of brief feeding bouts (4 min/hr), larvae spend the majority of their time resting on the “ceiling” inside their shelters (Weiss et al., 2003). In a kudzu patch, these shelters are easy to locate because the underside of leaves are much lighter in color than the tops of leaves. Thus, leaf shelters appear as white spots in characteristic locations on the edge of kudzu leaflets. For each collection date, kudzu patches were investigated thoroughly for as many shelters as could be located, and the entire leaflet containing a larva was collected and transported to the laboratory. Larvae from each site were then separated into four age groups based on the size of the insect head capsule. Larvae within an age group from each location were evenly distributed among plants in the eight blocks. Larvae were transferred using a small paintbrush, placed individually on one leaflet of a trifoliolate leaf, and were observed constructing new shelters almost immediately. Because caterpillars were collected weekly, most larvae put on plants were in early developmental stadia. The average number of larvae placed on each plant during the five week experimental period was  $48.3 \pm 1.9$  (mean  $\pm$  SEM).

In September, after field populations of *E. clarus* began to decline, larvae were no longer added and leaf cutting treatments were no longer applied. Plants remained untouched until the weeks of 23 October and 3 November, when above- and below-ground plant material were harvested for all plants. Above-ground biomass was collected by cutting off the top of the rootcrown at the soil surface to maintain all vine

connections, placing the plant material in large plastic trash bags, and storing it at 2 °C. Leaves were removed from vines, and placed in paper bags. In the laboratory, the rootcrown with all vines still attached was placed at one end of a long bench. All vines originating from the rootcrown were then stretched out along the length of the bench, and the number of shoot tips in each of the following length categories was counted: 0 to 2 m, 2 to 4 m, and 4 m or more. Additionally, the length of the ten longest vines from the rootcrown was measured individually. Because these measurements were made from the rootcrown to the longest shoot tip of intact plants (minus leaves), these measurements do not necessarily reflect the length of a single vine originating directly from the rootcrown (primary vine), but could have been higher order branches.

After measurements were made for intact plants, a primary vine was cut from the rootcrown, and all secondary branches were removed from that vine and placed to the side. The length of the primary vine was measured and the number of stem nodes counted. Then, tertiary vines were removed from secondary vines, and the length and number of stem nodes were recorded for secondary vines. This process was repeated for all branch categories. From these data we were able to determine the total vine length as the sum of individual vine lengths, average internode length, and number of branches. For each branch category (primary, secondary, etc.), we were able to determine the total length of vines, average vine length, average internode length and the proportion of vines in each category. Once measurements were taken, vines were placed in individually marked paper bags. Below-ground biomass was collected by digging a 1 m diameter circle with the base of the plant at the center. All roots with a diameter > 2 mm were collected, cleared of soil by washing with water and placed in

individual marked paper bags. Leaf, stem and root biomass in individual paper bags for each plant were dried in a greenhouse for several weeks before weighing.

Statistical analyses were performed using SAS (SAS Institute, 2008). Analysis of variance (ANOVA) by block and treatment was used to investigate biomass, the proportion of vines in each branch category, internode length, and vine length variables. A chi-square goodness of fit test was used to compare percent leaf biomass of plants (out of total above-ground biomass) in each treatment, with the null hypothesis that all three treatments had the same percent allocation to leaves compared to stems by the end of the season. ANOVA assumptions of normality and homoscedasticity were tested using the Shapiro-Wilk test in the UNIVARIATE procedure and Levene's test in the GLM procedure. Data that violated one or both of these assumptions were natural log transformed as  $\text{LN}(X)$ . Branch length, branch average internode length, and the number of vines in each length category that violated assumptions were natural log transformed as  $\text{LN}(X + 0.5)$ , as some recorded values were zero. To stabilize variance for proportion data, the percentage of vines in each branch category was arcsine-squareroot transformed because percentages fell outside the range of 30 to 70% (Snedecor and Cochran, 1967). If transformed data also violated ANOVA assumptions, the nonparametric Kruskal-Wallis test of the NPAR1WAY WILCOXON procedure was used. Data were ranked and an ANOVA (GLM procedure) was performed on the ranks. Tukey's test was used for mean separation.

## RESULTS

During the experiment, no measurements were made regarding the amount of biomass removed by damage treatments. However, by the end of the experiment it was obvious that simulated damage (75% leaf cutting) removed more leaf tissue than actual herbivory by *E. clarus*. Personal observations indicate that each larva was capable of consuming  $\leq 1$  leaf (three leaflets) during its development. For control plants,  $> 500$  leaves were produced during the season (total number of stem nodes, Table 3.1). Therefore, the addition of approximately 50 caterpillars per plant would produce roughly 10% leaf damage in the actual herbivory treatment.

For intact plants with vines still connected to rootcrowns, plants from all treatments had an average of eight to ten short vines (0 to 2 m long) and three to seven long vines (4 m or longer; Table 3.2). However, plants subject to simulated herbivory had an average of only three vines in the intermediate length category (2 to 4 m long), which was significantly fewer than the eight intermediate vines recorded for plants in the actual herbivory treatments (Table 3.2). The average length of the ten longest vines measured for intact plants was reduced from just over 4 m in the control and actual herbivory treatments to less than 2.5 m in the simulated damage treatments (Table 3.1). When the lengths of all individual vines per plant were summed, simulated herbivory halved the average total vine length compared to the control (Table 3.1). Average internode length was also significantly reduced by simulated herbivory, and a reduction of more than half was observed compared to the control (Table 3.1). For all damage types on intact plants, there were no differences for the total number of individual branches per plant (including primary, secondary, tertiary branches, etc.), or the total number of stem nodes per plant (Table 3.1).

Within a plant, the proportion of primary branches was significantly different for all treatments (Table 3.3). Control plants had the highest percentage of primary branches (15.5%), simulated herbivory the lowest (8.9%), and plants in the actual herbivory treatment had an intermediate proportion (11%). There were no differences in the proportion of branches for secondary, tertiary, or the combined number of quaternary and quinary branches (Table 3.3). Simulated herbivory reduced the average vine length of secondary branches (Table 3.4) and the total vine length of secondary branches (Table 3.5) compared to the control and actual herbivory. Average internode length did not differ for primary and quaternary vines, but was significantly reduced for secondary and tertiary branches in the simulated herbivory treatment compared to the control (Table 3.6). Figure 1 illustrates these differences in diagrammatic form for the control and simulated herbivory treatments, with numbers of branches, branch lengths, and internode length based on average values from the eight replicates.

Leaf, stem, and total above-ground biomass were significantly reduced by simulated damage compared to both the control and actual herbivory (Table 3.7). Control plants produced 2.2 and 2.9 times the amount of leaf and stem biomass, respectively, as plants subject to simulated herbivory (Table 3.7). There was no difference in the percentage of leaf biomass (out of total above-ground biomass) by treatment, and values ranged from 44 to 52% (Table 3.7). Root biomass was significantly reduced in the simulated damage treatment compared to the control, but plants subject to actual herbivory did not differ from either simulated damage or the control (Table 3.7). There was no difference in the root:shoot biomass ratio for plants in the three treatments (Table 3.7).

**Table 3.1.** Effect of actual and simulated herbivory on kudzu plants. Means  $\pm$  SEM in a column with the same letter are not significantly different (Tukey's test used for mean separation).

| Damage Type | N | 10 Longest Vines (m) |        | Total Vine Length (m) |                    | Average Internode Distance (cm) |     | Total Number of Branches |        | Total Number of Stem Nodes |                    |        |   |                    |        |   |
|-------------|---|----------------------|--------|-----------------------|--------------------|---------------------------------|-----|--------------------------|--------|----------------------------|--------------------|--------|---|--------------------|--------|---|
|             |   | Mean                 | SEM    | Mean                  | SEM                | Mean                            | SEM | Mean                     | SEM    | Mean                       | SEM                |        |   |                    |        |   |
| Control     | 8 | 4.12                 | ± 0.30 | A                     | 61.12              | ± 14.68                         | A   | 7.99                     | ± 0.99 | A                          | 75.0               | ± 14.3 | A | 507.9              | ± 99.9 | A |
| Actual      | 8 | 4.16                 | ± 0.21 | A                     | 73.75              | ± 22.45                         | A   | 9.15                     | ± 1.85 | A                          | 55.0               | ± 6.2  | A | 421.6              | ± 68.4 | A |
| Simulated   | 8 | 2.35                 | ± 0.22 | B                     | 31.61              | ± 10.81                         | B   | 3.69                     | ± 0.79 | B                          | 84.0               | ± 15.9 | A | 407.1              | ± 96.5 | A |
|             |   | $F_{2, 232} = 21.62$ |        |                       | $F_{9, 14} = 6.47$ |                                 |     | $F_{9, 14} = 11.81$      |        |                            | $F_{9, 14} = 1.58$ |        |   | $F_{9, 14} = 1.12$ |        |   |
|             |   | $P < 0.0001$         |        |                       | $P = 0.0102$       |                                 |     | $P = 0.0010$             |        |                            | $P = 0.2413$       |        |   | $P = 0.3546$       |        |   |

**Table 3.2.** Average number of vines in each length category per treatment. Means  $\pm$  SEM in a column with the same letter are not significantly different (Tukey's test used for mean separation).

| Damage Type | N | Length Category    |                    |                    |
|-------------|---|--------------------|--------------------|--------------------|
|             |   | 0 to 2 m           | 2 to 4 m           | 4 m plus           |
| Control     | 8 | 9.8 $\pm$ 1.70 A   | 7.0 $\pm$ 1.39 AB  | 5.1 $\pm$ 1.89 A   |
| Actual      | 8 | 8.4 $\pm$ 2.74 A   | 8.4 $\pm$ 1.98 A   | 7.4 $\pm$ 3.35 A   |
| Simulated   | 8 | 9.5 $\pm$ 1.39 A   | 3.0 $\pm$ 1.16 B   | 2.8 $\pm$ 1.54 A   |
|             |   | $F_{9, 14} = 0.11$ | $F_{9, 14} = 6.36$ | $F_{9, 14} = 1.85$ |
|             |   | $P = 0.8957$       | $P = 0.0108$       | $P = 0.1940$       |

**Table 3.3.** Percentage of branches in each category in response to actual and simulated herbivory. Means  $\pm$  SEM in a column with the same letter are not significantly different (Tukey's test used for mean separation).

| <b>Damage Type</b> | <b>N</b> | <b>% Primary</b>   | <b>% Secondary</b> | <b>% Tertiary</b>  | <b>% Quat/Quinary</b> |
|--------------------|----------|--------------------|--------------------|--------------------|-----------------------|
| Control            | 8        | 15.50 $\pm$ 1.43 A | 49.76 $\pm$ 5.22 A | 31.40 $\pm$ 4.69 A | 3.33 $\pm$ 1.69 A     |
| Actual             | 8        | 11.00 $\pm$ 0.69 B | 46.83 $\pm$ 4.17 A | 36.44 $\pm$ 3.61 A | 5.73 $\pm$ 2.73 A     |
| Simulated          | 8        | 8.90 $\pm$ 1.24 C  | 42.61 $\pm$ 4.41 A | 36.08 $\pm$ 5.10 A | 12.41 $\pm$ 5.86 A    |
|                    |          | $F_{9,13} = 29.57$ | $F_{9,13} = 0.53$  | $F_{9,13} = 0.27$  | $F_{9,13} = 1.47$     |
|                    |          | $P < 0.0001$       | $P = 0.6031$       | $P = 0.7699$       | $P = 0.2667$          |

**Table 3.4.** Average length of vines (m) in each category in response to actual and simulated herbivory. Means  $\pm$  SEM in a column with the same letter are not significantly different (Tukey's test used for mean separation).

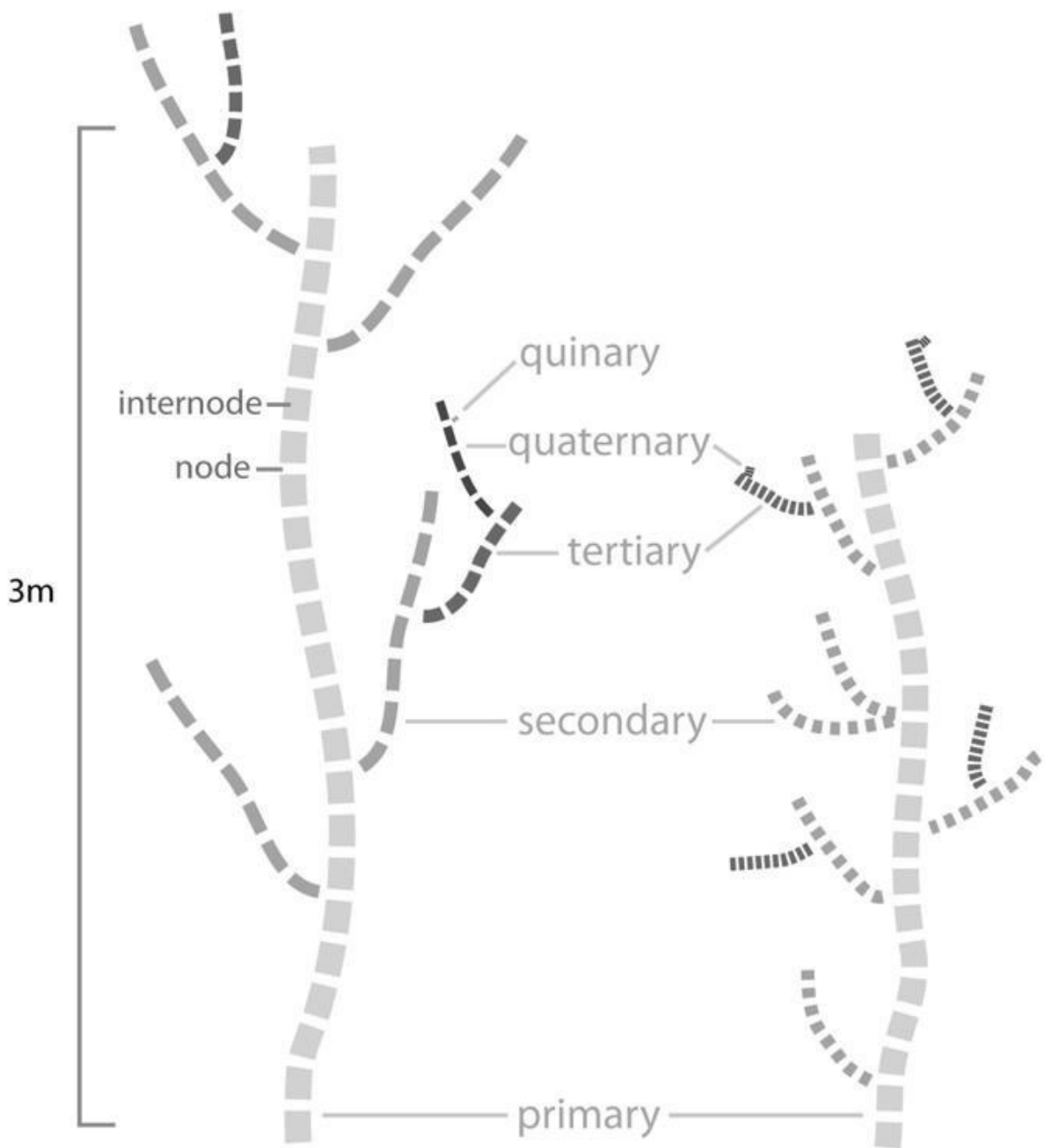
| <b>Damage Type</b> | <b>N</b> | <b>Primary</b>    | <b>Secondary</b>  | <b>Tertiary</b>   | <b>Quaternary</b> |
|--------------------|----------|-------------------|-------------------|-------------------|-------------------|
| Control            | 8        | 2.72 $\pm$ 0.86 A | 0.91 $\pm$ 0.21 A | 0.54 $\pm$ 0.17 A | 0.51 $\pm$ 0.37 A |
| Actual             | 8        | 2.63 $\pm$ 0.50 A | 1.16 $\pm$ 0.29 A | 0.79 $\pm$ 0.36 A | 0.23 $\pm$ 0.15 A |
| Simulated          | 8        | 1.73 $\pm$ 0.49 A | 0.32 $\pm$ 0.06 B | 0.21 $\pm$ 0.09 A | 0.03 $\pm$ 0.02 A |
|                    |          | $F_{9,13} = 1.07$ | $F_{2,20} = 8.41$ | $F_{2,20} = 3.30$ | $F_{2,20} = 0.91$ |
|                    |          | $P = 0.3717$      | $P = 0.0022$      | $P = 0.0577$      | $P = 0.4332$      |

**Table 3.5.** Total length of vines (m) in each category in response to actual and simulated herbivory. Means  $\pm$  SEM in a column with the same letter are not significantly different (Tukey's test used for mean separation).

| <b>Damage Type</b> | <b>N</b> | <b>Primary</b>                    | <b>Secondary</b>                  | <b>Tertiary</b>                   | <b>Quaternary</b>                 |
|--------------------|----------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Control            | 8        | 23.01 $\pm$ 6.68 A                | 26.21 $\pm$ 7.71 A                | 10.21 $\pm$ 4.68 A                | 0.52 $\pm$ 0.36 A                 |
| Actual             | 8        | 20.12 $\pm$ 4.87 A                | 33.94 $\pm$ 9.45 A                | 16.71 $\pm$ 7.62 A                | 2.52 $\pm$ 1.88 A                 |
| Simulated          | 8        | 11.32 $\pm$ 3.28 A                | 14.04 $\pm$ 5.48 B                | 5.69 $\pm$ 2.32 A                 | 0.44 $\pm$ 0.30 A                 |
|                    |          | $F_{9,13} = 1.15$<br>$P = 0.3461$ | $F_{9,13} = 7.43$<br>$P = 0.0071$ | $F_{9,13} = 1.68$<br>$P = 0.2238$ | $F_{2,20} = 0.21$<br>$P = 0.8144$ |

**Table 3.6.** Average internode length of vines (cm) in each category in response to actual and simulated herbivory. Means  $\pm$  SEM in a column with the same letter are not significantly different (Tukey's test used for mean separation).

| <b>Damage Type</b> | <b>N</b> | <b>Primary</b>                    | <b>Secondary</b>                  | <b>Tertiary</b>                   | <b>Quaternary</b>                 |
|--------------------|----------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Control            | 8        | 13.17 $\pm$ 2.15 A                | 7.11 $\pm$ 0.94 A                 | 6.20 $\pm$ 1.37 A                 | 4.18 $\pm$ 2.79 A                 |
| Actual             | 8        | 13.75 $\pm$ 1.29 A                | 9.22 $\pm$ 1.71 A                 | 5.55 $\pm$ 1.27 AB                | 2.43 $\pm$ 1.21 A                 |
| Simulated          | 8        | 9.70 $\pm$ 1.38 A                 | 4.27 $\pm$ 1.01 B                 | 2.36 $\pm$ 0.50 B                 | 0.70 $\pm$ 0.23 A                 |
|                    |          | $F_{9,13} = 3.16$<br>$P = 0.0764$ | $F_{9,13} = 8.59$<br>$P = 0.0042$ | $F_{9,13} = 4.83$<br>$P = 0.0270$ | $F_{2,20} = 0.24$<br>$P = 0.7890$ |



**Figure 3.1.** Diagrammatic rendering of an average vine from the control and simulated herbivory treatments, with number of branches, branch length, and internode length based on average measurements from eight plants per treatment. Simulated herbivory significantly reduced the proportion of primary vines, the average length of secondary vines, and the average internode length of secondary and tertiary vines.

**Table 3.7.** Effect of actual and simulated herbivory on kudzu biomass. Means  $\pm$  SEM in a column with the same letter are not significantly different (Tukey's test used for mean separation).

| <b>Damage Type</b> | <b>N</b> | <b>Leaf Biomass (g)</b>           | <b>Stem Biomass (g)</b>           | <b>% Leaf Biomass<sup>a</sup></b>   | <b>Total Above-ground Biomass (g)</b> | <b>Root Biomass (g)</b>           | <b>Root:Shoot Ratio</b>           |
|--------------------|----------|-----------------------------------|-----------------------------------|-------------------------------------|---------------------------------------|-----------------------------------|-----------------------------------|
| Control            | 8        | 205.00 $\pm$ 44.90 A              | 256.10 $\pm$ 92.35 A              | 48% A                               | 461.10 $\pm$ 135.53 A                 | 15.44 $\pm$ 1.71 A                | 0.06 $\pm$ 0.02 A                 |
| Actual             | 8        | 232.96 $\pm$ 62.54 A              | 250.46 $\pm$ 89.25 A              | 44% A                               | 483.43 $\pm$ 151.51 A                 | 13.14 $\pm$ 3.46 AB               | 0.05 $\pm$ 0.02 A                 |
| Simulated          | 8        | 95.04 $\pm$ 26.14 B               | 87.21 $\pm$ 31.04 B               | 52% A                               | 182.25 $\pm$ 57.03 B                  | 8.19 $\pm$ 1.20 B                 | 0.07 $\pm$ 0.02 A                 |
|                    |          | $F_{9,14} = 8.50$<br>$P = 0.0038$ | $F_{9,14} = 6.50$<br>$P = 0.0101$ | $\chi^2_2 = 0.0061$<br>$P = 0.9969$ | $F_{9,14} = 7.72$<br>$P = 0.0055$     | $F_{9,14} = 4.40$<br>$P = 0.0351$ | $F_{9,14} = 1.36$<br>$P = 0.2890$ |

<sup>a</sup> Percentage of total above-ground biomass that consisted of leaves.

## DISCUSSION

In this study, simulated herbivory reduced above- and below-ground biomass, total vine length, and average internode length of kudzu plants. In addition, the average length of the ten longest vines per plant, and the number of vines in the intermediate vine length category of 2 to 4 m was reduced by simulated herbivory. Differences in plant architecture included reductions in the proportion of all vines that were categorized as primary branches, reductions in average and total vine length of secondary branches, and reduced average internode length of secondary and tertiary branches in plants with simulated herbivory compared to the control.

Based on previous work demonstrating that the amount of tissue removed by herbivores may not accurately reflect losses to photosynthesis (Nabity et al., 2009), and that the extent of damage by herbivores may be six times greater than the amount of leaf material removed (Zangerl et al., 2002), we predicted that equal amounts of damage to plants would result in greater losses for the actual herbivory treatment than for the simulated herbivory treatment. However, in this study we were unable to produce comparable amounts of tissue loss for plants in the actual herbivory treatment, and did not find any impact of larval feeding by the silver-spotted skipper, *Epargyreus clarus*, on the measured plant parameters.

As noted, an average of over 500 leaves was produced by control plants during the 19 wk period from planting to harvest. While 75% of each newly emerged leaflet per leaf was removed by cutting in the simulated herbivory treatment, fewer than 50 insects were added to plants during the same time frame in the actual herbivory treatment, with insects capable of consuming only about one leaf each (personal observation). These low numbers of herbivores could have been exacerbated

by predation, as research specific to *E. clarus* has demonstrated that *Polistes* wasps are capable of learning how to locate and remove caterpillars from leaf shelters (Weiss et al., 2004). Although direct predation by *Polistes* wasps was not observed, the presence of these insects was noted during our study. Under natural insect densities in the field, predation of fourth instar larvae by *Polistes* wasps has been recorded at 30% (Weiss, 2003), and searching efficacy by wasps is thought to increase with higher larval densities (Weiss et al., 2004). Because larvae were added each week and concentrated within a relatively simple agricultural system in our study, this could have made prey-finding easier and more predictable for predators to remove caterpillars, thereby reducing overall foliage consumption.

In addition to low herbivore numbers, native insects such as *E. clarus* may not be particularly adapted to feed on kudzu, and may exhibit reduced foliage consumption on this exotic plant compared to its natural hosts. For example, *Pseudoplusia includens* (Walker), the soybean looper, is a native, generalist caterpillar that feeds on a variety of legume species, including soybean and kudzu (Kidd and Orr, 2001, and references therein). In feeding experiments with *P. includens*, larvae fed on kudzu had lower survival, slower development rates (including supernumerary molts), and reduced pupal weights compared to larvae that fed on soybean (Kidd and Orr, 2001). Although no differences were found in the average foliage consumption by insects on the two plant species, differences at the population level would be expected based on a 30% reduction in insect survival from feeding. If the same is true for *E. clarus*, reduced feeding combined with the effects of predation by *Polistes* wasps could have dramatically reduced already low numbers and feeding damage by *E. clarus* in the field.

While previous studies examining the response of an invasive weed to simulated and actual herbivory have relied on natural enemies from the plant's native range to impose damage (Schat and Blossey, 2005), the lack of host specific natural enemies of kudzu in the United States required us to use a surrogate species for the actual damage treatment. *Epargyreus clarus* was chosen for several reasons, including its prevalence in local kudzu patches, and the ease with which it can be identified and collected in the field. Furthermore, *E. clarus* is a native insect that was unlikely to produce nontarget damage, especially for crops in adjacent fields such as soybean. However, based on low numbers and feeding by *E. clarus* in our experiment, we recommend future studies utilize more voracious insects that can be reared in the laboratory in large quantities, such as *Spodoptera frugiperda* (J.E. Smith).

The ability to outcompete native vegetation for light resources is a common explanation for the success of invasive vines such as kudzu. Imported natural enemies for biological control can disrupt this competitive advantage by not only shifting resource allocation away from growth and toward defense (Blossey and Nötzold, 1995), but also by changing plant architecture (Schat and Blossey, 2005). For example, melaleuca plants subject to simulated (Silvers et al., 2008) and actual herbivory (Pratt et al., 2005) produced higher numbers of branches, but overall branch lengths were reduced compared to undamaged plants. While a typical melaleuca plant enhances light exposure compared to neighboring vegetation by growing tall and narrow, plants subject to herbivory are shorter and have a bushy habit (Silvers et al., 2008). This has also been shown for invasive vine species, such as *Persicaria perfoliata* (L.) H. Gross (mile-a-minute weed), where the loss of apical dominance and resulting increases in branching can prevent plants from maximizing light exposure,

and decrease the competitive ability of the plant (Hough-Goldstein et al., 2008). For kudzu, leaf damage that decreases overall vine lengths will have similar effects on the plant's ability to compete for light resources. Furthermore, reductions in the internode length of secondary and tertiary vines might decrease the plant's ability to form roots at stem nodes, thereby reducing the plant's primary mechanism of reproduction. Thus, the observed changes in plant architecture might have effects that exceed those documented here.

Considering the history of kudzu as an invasive species in the United States, it is surprising that detailed information on the growth of individual plants is lacking. The work accomplished to date has examined above-ground biomass production from 0.5 m<sup>2</sup> quadrats (Abramovitz, 1983; Wechsler, 1977) as well as from large 15.3 by 30.5 m plots within established kudzu patches (Terrill et al., 2003). One study explicitly measured vine elongation rates (Wechsler, 1977), but results were derived from only five consecutive days of data collection in a single kudzu patch. Thus, commonly reported growth rates of individual vines ranging from 10 to 30 m a season, with average internode lengths of 30 to 60 cm (Mitich, 2000), should be considered anecdotal, but not unrealistic. In the current study, plants were grown from seed in a greenhouse and transplanted to an agricultural setting in the northern part of kudzu's range. Despite these artificial conditions and a short growing season in the field of only 19 wks before harvest, one newly transplanted kudzu plant produced three primary vines over 11 m, and two of those vines were longer than 12 m. Therefore, especially near the center of kudzu's invasive range where the growing season is extended, vines may reach 30 m in one year of growth. In other species with clonal growth habits, research has shown that rooted stem nodes can contribute to

overall plant health by acquiring resources with adventitious roots (Roila et al., 2010). Therefore, enhanced vine growth for kudzu is expected for plants in established, long lived patches, where physiological integration maintains connections between rooted stem nodes.

An important goal of classical weed biological control programs is to reduce the competitive ability of invasive plants (Müller-Schärer and Schaffner, 2008). Compared to control plants, simulated damage produced 40% less above-ground biomass, and root weight was reduced by 47%. The total vine length was shortened by 48%, and the average length of the ten longest vines dropped 43%. In natural situations, these changes can reduce the ability of kudzu to compete for light and other important resources by affecting the plants climbing habit. In addition, these changes may have other consequences for plants that have not yet been documented, such as reduced reproduction or decreased survival through the winter if allocation of resources is shifted to compensate for biomass loss. Therefore, while we were unable to directly compare simulated and actual herbivory to verify that biological control agents will impact the plant in natural situations, the ecological data obtained here indicates the potential for leaf damage to alter plant growth and architecture.

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