

Response of Kudzu (*Pueraria montana* var. *lobata*) Seedlings and Naturalized Plants to Simulated Herbivory

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We studied the response of naturalized kudzu plants to simulated herbivory at three locations: Delaware (DE), Pennsylvania (PA), and North Carolina (NC). At the DE and PA sites, plant mortality after the first yr was 14 and 50%, respectively, and was highest for plants that had a small starting root crown size. At both sites, 50 and 75% leaf and shoot clipping, and drilling one or two large holes from the root crown into roots, had no effect on aboveground biomass. In NC, all plants survived for 3 yr. Plants subject to 50% vine removal at this site showed significant decrease in aboveground biomass compared to the control, but 50% leaf cutting and root drilling had no effect. In the greenhouse, kudzu seedlings grown in 60 and 100% light compensated for 50% leaf removal, but 75% damage reduced aboveground biomass. Plants survived for 1 to 2 mo in 0% direct light, but only one of 53 plants survived to the end of the experiment. Results suggest that established kudzu plants are able to compensate for biomass removal, seedlings can survive for several weeks without light, and that effective biocontrol might require more than 2 to 3 yr of continuous damage.

Nomenclature: Kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & S. M. Almeida.

Key words: Classical biological control, light, seedlings, simulated herbivory.

Invasive, exotic species threaten the health and proper functioning of natural ecosystems worldwide (Mack et al. 2000), and are second only to habitat destruction in their impacts on 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], which was intentionally introduced to the United States in 1876 at the Centennial Exposition in Philadelphia (Mitich 2000) and was extensively planted for erosion control in the 1930s and 1940s (Miller and Edwards 1983). Kudzu now infests an estimated two to seven million acres (Britton et al. 2002), and is implicated as a cause of increased ozone pollution (Hickman et al. 2010) in addition to likely impacts on native plant biodiversity. Furthermore, the range of this weed is predicted to increase with global climate change (Jarnevich and Stohlgren 2009).

As early as the 1950s, kudzu was recognized as a weed, yet large-scale control of the plant remains a problem. Mechanical removal using machinery is difficult, because 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) by competing with them for light resources (Everest et al. 1991). Although time- and labor-intensive, removing individual plants by cutting below the root crown can provide complete control (Kudzu Coalition 2010; M. J. Frye, personal observation).

For large-scale infestations, kudzu control is typically accomplished with multiple 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). As with 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 herbicide application to mechanically

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Management Implications

Kudzu is an exotic, invasive weed that covers two to seven million acres in the southern United States. As part of a biological control research program targeting this weed, we clipped leaves, removed or clipped stems, and drilled holes in roots to determine what kind of damage would reduce plant growth and/or survival. Near the edge of kudzu's invasive range (Delaware and Pennsylvania), many plants died following the first year, even where damage treatments were not applied. Near the center of the range (North Carolina), only plants that had half of their vines removed (simulating severe damage by stem-boring beetles) had reduced growth, and none of the plants died. In a greenhouse study, almost all kudzu seedlings grown in complete shade died after 80 d. When leaves were clipped, plants grown under low light conditions were more likely to die than those in full sun. Results suggest that biocontrol alone might not be sufficient to control kudzu, especially near the center of its range. Therefore, field practitioners should consider integrating multiple control tactics to stress the plant, including mechanical and chemical controls, or competition techniques such as planting canopy species with dense foliage. If kudzu patches in full sun are cleared, practitioners should implement additional management techniques such as revegetation to limit the recruitment of new kudzu plants from the seed bank.

cut root crowns (Thomas 2000), or burning combined with reforestation (Harrington et al. 2003), have been shown to enhance suppression provided by herbicides. Recent research has explored the potential use of the fungal pathogen *Myrothecium verrucaria* (Alb. & Schwein) Ditmar:Fr. as a bioherbicide to kill kudzu (Anderson and Hallett 2004; Boyette et al. 2008).

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). Accidental insect introductions can impact target weeds similarly, but often pose risks to nontarget species. The bean plataspid *Megacopta cribraria* F., for example, is a kudzu-feeding insect native to Asia that was first found in the US in 2009 (Suiter et al. 2010). Earlier natural enemy surveys in China determined that this insect was not suitable for biological control due to its broad host range (Sun et al. 2006). Following its accidental introduction, tests have confirmed that *M. cribraria* can feed on several native and economically important legumes by sucking sap from stems, petioles, and leaves, with the potential to damage fruit (Zhang et al. 2012). Although the insect is capable of reducing the growth of kudzu, nontarget feeding is assumed to outweigh any benefit of this introduced species.

Where biological control is intentionally applied, only very host-specific organisms are imported. This reduces risks and makes biocontrol safer than large-scale herbicide applications or mowing. Unfortunately, time and funding might not be available to develop this method for a particular weed species, 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* Selman and Vogt, introduced for biological control of alligator weed [*Alternanthera philoxeroides* (Martius) Grisebach] successfully reduced weed stands in aquatic, but not terrestrial habitats (Sun et al. 2009, and references therein). Sun et al. (2009) conducted a full 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 (e.g., Manrique et al. 2008).

As part of a biological control program targeting kudzu in the United States (Britton et al. 2002), we investigated the growth response of individual plants to different types of simulated herbivore damage. This study was conducted over several seasons in established kudzu patches near the center (North Carolina, NC) and edge (Delaware, DE and Pennsylvania, PA) of kudzu's invasive range. Leaf, root, and shoot treatments were selected to simulate the damage imposed by important insect feeding guilds observed on the plant in China (Sun et al. 2006). This study compliments a previously reported study conducted in farm fields in both NC and DE, which showed that 75% leaf cutting consistently reduced biomass in both locations (Frye et al. 2012). In the current study, field experiments were supplemented with a greenhouse study to examine emergence, survival, and the growth response of kudzu seedlings to different light exposures in combination with leaf cutting treatments.

Materials and Methods

DE and PA Field Experiments. Treatments were applied to established kudzu plants at sites in 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). The DE site was characterized by sandy soil, with very little organic matter. Except for early morning hours, plants were in direct sunlight most of the day. Low- to medium-sized herbs were

common at the site, especially bull thistle [*Cirsium vulgare* (Savi) Ten.]; mugwort (*Artemisia vulgaris* L.); mile-a-minute weed or devil's tearthumb [*Polygonum perfoliatum* L., syn. = *Persicaria perfoliata* (L.) H. Gross]; and eastern poison-ivy [*Toxicodendron radicans* (L.) Kuntze]. In PA, kudzu plants were on an east-facing slope in a light gap surrounded by mesic woods. The soil was characteristic of the Piedmont with high organic content (McAvoy and Bennett 2001). Several other invasive plants were abundant at the site, including multiflora rose (*Rosa multiflora* Thunb. ex Murr.), Japanese stiltgrass [*Microstegium vimineum* (Trin.) A. Camus], and mile-a-minute weed. Trees such as tulip poplar (*Liriodendron tulipifera* L.) and black walnut (*Juglans nigra* L.) surrounded the patch on all sides and shaded the plants variably throughout the day.

In early May 2008 when new foliage was just beginning to emerge, 36 individual kudzu plants, each with a root crown at the plant/soil interface, were located at each of the two sites. Surrounding vegetation was cleared to expose bare soil, and woody overwintered vines were removed by cutting so that only new growth would originate from the root crown. Each plant was enclosed in a cylindrical metal wire cage (1 m [3.3 ft] diameter, 0.92 m [3 ft] 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 the 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 root crown using calipers (Scherr-Tumico Industries, Inc., St. James, MN). The area of an ellipse ($\pi \cdot 0.5 \text{ length} \cdot 0.5 \text{ width}$) was calculated to estimate the size of each root crown.

Treatments were applied to simulate defoliation, root feeding, and shoot clipping. One treatment was randomly assigned to each plant at a site, and included two levels of each damage type: cutting 50% and 75% of each leaf; clipping off the last 15 cm [6 in] from 50% and 75% of the total number of shoots; drilling one (Root 1) or two (Root 2) holes from the root crown into the roots; 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). Leaf damage was applied to all leaves on the plant in both the 50% and 75% leaf cutting treatments during the first week of study, and to new, fully expanded leaves each week thereafter. Fifty percent leaf damage was achieved by cutting the right side of each leaflet without damaging the midvein. Seventy-five percent leaf cutting removed the right half of each leaflet per leaf, and then cut the remaining leaflet in half so that only the lower left quarter of each leaflet remained intact. For root damage, the total depth, diameter of the drill bit, location of drilling, and weekly incremental increase in drilling depth were based on damage reported for a root-feeding

cerambycid beetle in China (Sun et al. 2006). The root treatment was applied using a 0.64-cm drill bit; depth of drilling started at 0.02 cm in week 1 and increased by a factor of 1.3 to a total depth of 10.24 cm by week 16. For the shoot clipping treatment, the length of all shoots was compared to a 15-cm ruler from the tip back toward the root crown, the total number of shoot tips over 15 cm counted, and the last 15 cm of 50% or 75% of the total number of shoot tips were removed. This treatment mimicked the damage produced by a curculionid weevil (Frye et al. 2007) and two species of attelabid beetles (Sun et al. 2006). Treatments were applied to plants for 16 wk starting on June 9, 2008. Aboveground biomass of all plants was harvested around the time of the first killing frost, the week of October 19, 2008. Plant material was placed in large paper bags, dried in a greenhouse for several weeks, and weighed (Brainweigh B15000D, Ohaus Corp., Pine Brook, NJ).

In 2009, the study was repeated in DE and PA with the same plants. Overwintering survival to 2009 was recorded, and surviving plants received the same damage treatments as in 2008, weekly for 16 wk starting on June 8, 2009. In October 2009, aboveground plant biomass was harvested and dry weight was determined for all plants from both sites.

NC Field Experiment. Treatments in NC included weekly cutting of 50% of each leaflet per leaf; drilling a 10.24 cm deep hole from the root crown into the roots; removing 50% of the total number of vines greater than 15 cm by clipping at the base of the plant, which simulated damage by the stem-feeding insect guild (Sun et al. 2006); and a no-damage control. Treatments were applied to established kudzu plants at a site in Butner, NC (36°09'58.09"N, 78°47'03.20"W), beginning in 2007. The soil at this site is classified as Georgeville silt loam, which is well-drained and contains a surface layer of brown silt loam, underlain by yellow-red silt loam, then red clay (Hayes 2006). The site was relatively flat and under full sun exposure, bounded by a small cemetery, a corn field across a dirt farm road, pine trees, and a two-lane road. Few plants other than kudzu were present, and a mower and weed eater were used periodically to keep the surrounding kudzu from engulfing the designated plants. Twenty-four root crowns were isolated from surrounding vegetation, measured (length and width to determine area of an ellipse) and designated as small (3.47 to 10.31 cm²), medium (13.33 to 25.72 cm²), or large (31.49 to 55.29 cm²). Two of each of the three root crown size designations were assigned randomly to each treatment ($N = 6$ per treatment). As in DE and PA, all woody vines attached to the target root crowns were removed at the start of the experiment.

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 once weekly for 16 wk each year (June 19 to October 3, 2007; June 4 to September 24, 2008; June 4 to September 24, 2009). At the end of each season, aboveground plant biomass was harvested and dried for approximately 4 d in a gas-fired curing barn with strong air circulation at 62.7 C prior to weighing.

Greenhouse Study on Impact of Light and Defoliation.

Two experiments were conducted simultaneously 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 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). The seeds were kept separate and used for different experiments, each with its own controls, because of known genetic differences in kudzu throughout the United States (Pappert et al. 2000), and likely genetic differences in populations from DE and NY. Pods from both sites 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 by 7.6 cm square pots with 25 pots per tray. Two seeds were sown per pot for NY seeds on February 25, 2009, and one seed per pot for DE seed on March 14, 2009. Immediately after seeds were sown, two trays (total of 50 pots) from each seed source were placed on benches in each of the three light treatments. Pots were watered by hand as needed, and fertilized once weekly with 200 parts per million (ppm) nitrogen solution. Percent emergence (appearance of the shoot above the soil) of the total number of seeds planted was determined on April 7, 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 April 14, 2009, prior to leaf cutting, was used to

determine percent survival of plants in each treatment for the next 8 wk.

Treatments were assigned randomly to plants the week of April 13, 2009 by flipping a coin, and included 50% leaf cutting for DE seedlings, 75% leaf cutting for NY seedlings, and equal numbers of no-damage controls for both seed sources in each light treatment. Treatments were applied to all leaves the first week of study and to newly expanded leaves thereafter. Spinosad (Conserve SC, Dow AgroSciences LLC, Indianapolis, IN) was applied to all plants starting April 16 and once weekly thereafter for control of thrips.

On May 11, 2009, individual pots were removed from trays and distributed evenly on benches to prevent overlap of foliage and potential shading. All aboveground biomass of plants was harvested the week of July 20, 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. Plant material was dried in a greenhouse for several weeks, and then weighed to determine aboveground biomass. Plants that did not survive were not included in this analysis.

Statistical Analysis. Statistical analyses were performed using SAS (SAS 2008). For field experiments, regression was used to assess the relationship between root crown size and plant biomass, and analysis of variance (ANOVA) was used to compare root crown size between treatments. Analysis of covariance (ANCOVA) was used to assess plant mortality, with root crown size as a covariate for the DE and PA sites. Plant biomass was investigated using repeated measures ANOVA. For the greenhouse study, two-way ANOVA was used to assess biomass, vine length, and internode length for light by damage treatment interactions. The Shapiro-Wilk and Levene's tests were used to assess normality and homoscedasticity of the data, respectively. Data that violated one or both of these ANOVA assumptions were natural log-transformed. If transformed data also violated ANOVA assumptions, the nonparametric Kruskal-Wallis test was used, with ranked data analyzed by ANOVA. Field biomass data were not transformed because repeated measures ANOVA is robust to heteroscedasticity. All other data transformations are reported with results. 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 were arcsine-squareroot transformed because the proportion of surviving plants was outside the range of 30 to 70% (Snedecor and Cochran 1980). Untransformed data are presented in tables and figures.

Results and Discussion

Impacts of Treatments at Field Sites. Initial root crown sizes ranged from 1.4 to 73.0 cm² in DE and 1.4 to

Table 1. Average crown size per site, and relationship with aboveground biomass after the first year of study.

Location (year initiated)	Crown size ^a	N	Aboveground biomass correlation			
			F	df	P	r ²
DE (2008)	30.6 ± 2.9 a	36	7.70	1, 34	0.0089	0.1607
NC (2007)	22.5 ± 3.1 ab	24	0.86	1, 22	0.3639	-0.0061
PA (2008)	16.7 ± 2.3 b	36	4.30	1, 34	0.0457	0.0863

^a Means ± SEM followed by the same letter are not significantly different ($F_{2, 91} = 7.60$, $P = 0.0009$ for natural log transformed data; Tukey's test used for mean separation).

51.2 cm² in PA. Average crown size was significantly larger at the DE site than the PA site (Table 1). In NC, root crown size ranged from 3.5 to 55.3 cm², with average root crown size intermediate between values for DE and PA (Table 1). There was no difference in initial root crown size by treatment at any site (DE: $F_{6, 29} = 0.16$, $P = 0.9852$; PA: $F_{6, 29} = 0.45$, $P = 0.8371$; NC: $F_{3, 20} = 0.03$, $P = 0.9913$).

A significant correlation between initial root crown size and aboveground biomass at the end of the first yr (including all treatments) was detected at both the DE and PA sites, with low r^2 values (Table 1). This weak correlation indicates that plant productivity might be determined in part by root crown size. This finding is consistent with current knowledge regarding kudzu growth, specifically that the plant allocates carbohydrates to roots at the end of the growing season (Wechsler 1977). Increases in the size of the root crown might be coupled with root expansion in the fall of each year. Therefore, to remove existing among-plant variation within a site, root crown size was included as a covariate in analyses of plant growth parameters. Correlation between aboveground biomass and root crown size was not found at the NC site (Table 1).

Plant survival in NC from 2007 to 2009 was 100%, whereas 86% of plants at the DE site and 50% of plants at the PA site survived from 2008 and 2009. Plant survival was significantly lower in PA than DE ($\chi^2_1 = 7.35$, $P = 0.0067$), and pooled data from both sites showed that plants that died had smaller root crowns (average size: 16.4 ± 2.7 cm²) than those that survived (average size: 26.7 ± 2.5 cm²; $F_{1, 70} = 6.10$, $P = 0.0160$). There was no significant difference in plant survival by treatment for either DE ($\chi^2_6 = 1.53$, $P = 0.9575$) or PA ($\chi^2_6 = 3.6811$, $P = 0.7197$).

Kudzu is a perennial vine that spreads primarily by vegetative reproduction in the United States. 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). In other systems, root crown size is thought to vary with plant age, resource availability, or herbivore attack. For example, *Cirsium altissimum* (L.) Hill (Asteraceae) is a perennial herbaceous thistle that produces

a basal rosette or root crown (Guretzky and Louda 1997). When insecticide treatments exclude herbivores from the plant, root crown size increases incrementally throughout the season. However, plants subject to herbivory by multiple herbivores produce less aboveground biomass and have a reduced crown size (Guretzky and Louda 1997). In the current study, variation in kudzu root crown size could be a product of patch age (time since establishment) and/or site management history, with older plants exhibiting larger crowns. Furthermore, allocation to roots can be resource-driven in nutrient-poor environments (Gleeson and Tilman 1990), and this too could explain the larger crown sizes at the sandy, low-nutrient DE site compared to the PA site. In any case, plants with smaller crowns will have fewer resources, which explains the inverse relationship between crown size and plant mortality in DE and PA. More research is needed to understand the relationship between crown size and plant productivity in naturalized kudzu patches.

Aboveground biomass of plants from DE and PA did not differ significantly by treatment in 2008 or 2009 (Figures 1 and 2). Average aboveground biomass per site was about twice as high in DE as in PA in 2008 (DE, 60.7 ± 5.3 g [2.1 ± 0.2 oz], $N = 36$; PA, 30.4 ± 3.0 g, $N = 34$; $F_{13, 56} = 20.61$, $P < 0.0001$) and 2009 (DE, 32.4 ± 3.8 g, $N = 31$; PA, 15.2 ± 2.3 g, $N = 18$; $F_{13, 35} = 9.04$, $P = 0.0049$). Plant biomass was significantly reduced in 2009 compared to 2008 at both DE (Figure 1) and PA (Figure 2).

At the NC site, biomass was not affected by root drilling compared to the control in any year (Figure 3). In addition to direct damage from feeding, herbivore-induced wounds in the root zone could indirectly affect plant survival and growth by serving as infection sites for pathogens and other microorganisms (Hunter 2001), but this was not observed in our experiment. The 50% leaf cutting treatment also did not affect biomass in NC (Figure 3), whereas the 50% vine removal treatment significantly reduced aboveground biomass compared to both the control and root drilling treatments in 2007 and 2008 (Figure 3). This treatment was much more drastic than the 15-cm shoot clipping treatment applied in DE and PA, but might not be unrealistic, because in China more than 50% of vines were

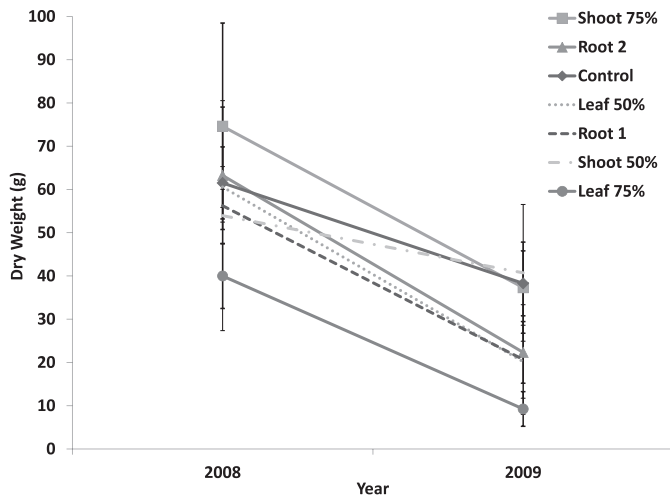


Figure 1. Aboveground biomass of DE plants in 2008 and 2009 (means \pm SEM; $N = 9$ for Control; 5 for Leaf 75%, Root 2, Shoot 75%; 4 for Leaf 50%, Root 1, Shoot 50%). Plants produced significantly less biomass in 2009 compared to 2008 ($F_{1, 29} = 31.03$, $P < 0.0001$). Damage treatments had no effect ($F_{6, 28} = 1.52$, $P = 0.2091$), and there was no interaction between treatment and year ($F_{6, 29} = 0.66$, $P = 0.6824$).

found to be damaged by cerambycid and buprestid stem-boring beetles, and damage levels as high as 80% were reported at some sites (Sun et al. 2006). In contrast to the results from DE and PA, plant biomass in NC increased significantly over time (Figure 3).

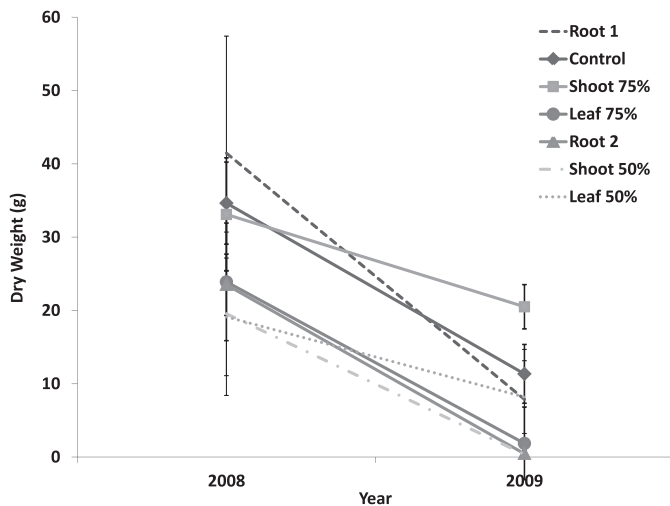


Figure 2. Aboveground biomass of PA plants in 2008 and 2009 (means \pm SEM; $N = 9$ for Control; 5 for Leaf 75%, Root 2, Shoot 75%; 4 for Leaf 50%, Root 1, Shoot 50%). Plant biomass was lower in 2009 than in 2008 ($F_{1, 29} = 47.83$, $P < 0.0001$). No differences due to treatment ($F_{6, 28} = 1.89$, $P = 0.1182$) and no treatment by year interactions ($F_{6, 29} = 0.91$, $P = 0.2790$) were detected at this site.

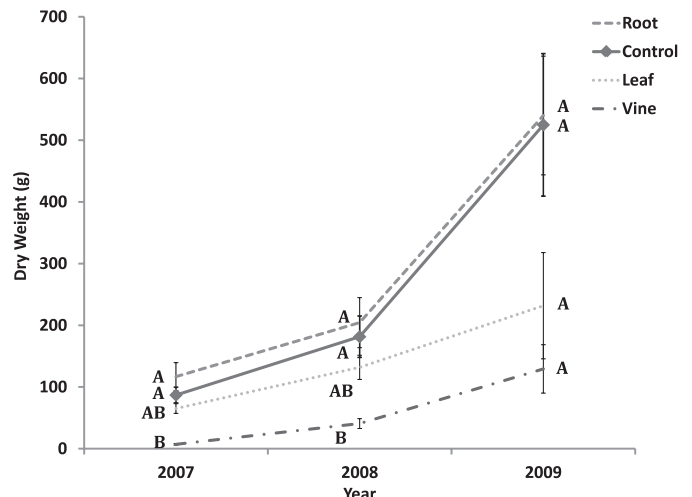


Figure 3. Aboveground biomass of NC plants from 2007 to 2009. Within a year, means (\pm SEM) with the same letter are not significantly different ($F_{3, 19} = 7.98$, $P = 0.0011$; $N = 6$ for all treatments and years; Tukey's test used for mean separation). Plant biomass increased significantly over time ($F_{2, 19} = 26.17$, $P < 0.0001$), and there was no year by treatment interaction ($F_{6, 24} = 2.08$, $P = 0.0942$).

In general, kudzu plants in this study showed no difference in their response to damage treatments that removed leaf, shoot, or root biomass, except for the NC treatment where 50% of all vines over 15 cm long were completely removed each week. One explanation for these results is plant compensatory response to herbivory, which has three potential outcomes (Maschinski and Whitham 1989). The first is termed undercompensation, and causes detrimental losses when plants are unable to replace tissue or resume equal productivity following herbivore damage. Complete compensation occurs when plants adjust productivity to yield no differences in biomass or seed production compared to undamaged controls, and overcompensation is when plants produce more biomass or seed as a result of herbivory when compared to control plants (Maschinski and Whitham 1989). With the exception of the 50% vine removal treatment in NC, kudzu plants in the current experiment were able to completely compensate for tissue lost to simulated leaf, root, and shoot damage treatments. This result was not unexpected because kudzu has a high allocation of resources to aboveground biomass (Forseth and Innis 2004), and carbohydrates stored in large, tuberous roots can be used for plant growth (Forseth and Teramura 1986). Thus, plants in DE, PA, and in NC (excluding the vine removal treatment) could have used stored root resources to overcome differences in biomass removal based on applied damage treatments and levels.

The lack of effects of root drilling in this experiment was surprising. Research in other systems has shown that

Table 2. New York plant response to light and cutting treatments.^a

Treatment	<i>N</i>	Vine length	Internode distance	Aboveground biomass
	% survival ^b	cm	cm	g
100% Light, Control	8 (100)	80.9 ± 10.9 a	2.6 ± 0.3 a	5.9 ± 0.4 a
100% Light, Leaf 75%	7 (70)	30.9 ± 10.5 bc	0.7 ± 0.2 b	2.7 ± 0.6 bc
60% Light, Control	6 (60)	45.2 ± 7.7 ab	2.5 ± 0.6 a	2.9 ± 0.2 b
60% Light, Leaf 75%	7 (64)	15.0 ± 5.2 c	0.6 ± 0.1 b	1.1 ± 0.4 c
0% Light, Control	0 (0)	—	—	—
0% Light, Leaf 75%	0 (0)	—	—	—

^a Means ± SEM followed by the same letter within a column are not significantly different ($P > 0.05$, ANOVA, Tukey's test used for mean separation).

^b *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 ($\chi^2_1 = 0.0657$, $P = 0.7977$).

simulating root damage is difficult (Blossey and Hunt-Joshi 2003), and this was true in our study. Our treatment was designed to simulate burrowing by natural enemies observed in China (Sun et al. 2006), but was complicated by an inability to drill directly from the root crown into roots of selected plants. Consequently, damage in several cases could be more accurately described as root crown damage. In addition, because treatments were assigned randomly, some plants had few, or small roots extending from the root crown into the soil at the start of the experiment, and drilling severed the connection between the root crown and roots in two plants in PA. Because roots alone cannot regenerate aboveground plant parts without the meristematic tissue present in root crowns, this resulted in death of the two plants. In the field, this result might be unrealistic because reports from 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 attacked by

herbivores that infest roots in China. 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.

Greenhouse Study on Impact of Light and Defoliation.

The observation that there was higher plant mortality, lower biomass, and smaller initial crown sizes at the shaded PA site compared to the more open DE site and the full-sun NC site in the field portion of this study suggested the possibility that light exposure might have important effects on kudzu growth, which might interact with herbivore feeding damage. The greenhouse study served as an initial test of this hypothesis.

Seedlings from both seed sources emerged from soil 5 to 6 d after planting. Percent emergence for the 0, 60, and 100% light treatments was 23, 24, and 21%, respectively, for NY seed ($N = 100$ for each light treatment), and 88,

Table 3. Delaware plant response to light and cutting treatments.^a

Treatment	<i>N</i>	Vine length	Internode distance	Aboveground biomass
	% survival ^b	cm	cm	g
100% Light, Control	24 (100)	93.1 ± 6.4 a	2.7 ± 0.2 b	5.8 ± 0.3 a
100% Light, Leaf 50%	19 (95)	87.7 ± 8.4 a	2.0 ± 0.2 b	5.7 ± 0.3 a
60% Light, Control	22 (100)	89.1 ± 7.3 a	3.6 ± 0.3 a	4.4 ± 0.3 b
60% Light, Leaf 50%	17 (77)	72.7 ± 6.7 a	2.6 ± 0.3 b	3.5 ± 0.2 b
0% Light, Control	1 (5)	7.0 ± 0.0	1.2 ± 0.0	0.2 ± 0.0
0% Light, Leaf 50%	0 (0)	—	—	—

^a Means ± SEM followed by the same letter within a column are not significantly different ($P > 0.05$, ANOVA, Tukey's test used for mean separation).

^b *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 ($\chi^2_2 = 0.1840$, $P = 0.912$).

88, and 94%, respectively, for DE seed ($N = 50$ for each light treatment). Plants in the 0% light treatment from both seed sources were etiolated, had darker leaves than plants in either the 60 or 100% light treatments, and stopped growing at a height of approximately 5 cm. There were no obvious differences in the appearance of plants grown in 60% vs. 100% direct light. 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).

In the experiment using seedlings from the NY seed source, plants in 0% direct light did not survive after May 12 (76 d after planting), regardless of whether or not 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 2). Across light treatments, 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 aboveground biomass ($F_{3, 24} = 23.99$, $P < 0.0001$) compared to control plants (Table 2). Cut plants in 60% light had significantly reduced vine lengths and aboveground biomass compared to control plants in either light treatment (Table 2).

Overall survival of seedlings from the DE seed source was 65%. There were no differences in the proportion of plants surviving to the end of the season by cutting treatment, but all control plants in 60% and 100% direct light survived (Table 3). A single control plant in 0% direct light survived, but was not included in subsequent analysis because all other DE plants in 0% light had died by June 2 (80 d after planting). Total vine length for DE seedlings did not differ by cutting treatment ($F_{3, 78} = 2.08$, $P = 0.1097$ for ranked data; Table 3). Average internode length 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 produced more aboveground biomass than plants in 60% light, regardless of cutting treatment ($F_{3, 78} = 18.26$, $P < 0.0001$; Table 3).

Results from these two experiments demonstrate the importance of light environment to kudzu seedling establishment, with almost complete mortality of seedlings grown in conditions of 0% direct light and generally lower biomass of plants grown in 60% light, especially in the 75% leaf cutting treatment (NY plants). These experiments also addressed a common misconception regarding the invasiveness of kudzu, i.e., that the plant is not capable of producing viable seed in the United States (Mitich 2000). Although seed coat dormancy in general is known for leguminous plant species (Quinlivan 1971), the results from these experiments 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 the field research sites in DE and PA, seedlings were observed midsummer in areas of disturbed soils, specifically on paths made to access plants, as well as within cages (M. J. Frye, personal observation). However, results from the current study indicate that, despite 98% mortality in 0% direct light, seedlings were able to survive for 1 to 2 mo after emergence in this condition. This has important implications for kudzu management, because plant removal from infested sites with herbicides or other control methods could result in recruitment of kudzu seedlings or other exotic invasive species, creating an “invasive species treadmill” (Thomas and Reid 2007).

Implications for Biological Control. Classical biological control has become an important tool in the management of invasive weed species in natural areas (Van Driesche et al. 2010). Host-specific natural enemies offer several advantages over mechanical and chemical control, including the ability of these organisms to spread, self-perpetuate, and offer long-term or sustained control (Van Driesche et al. 2008). Unfortunately, the introduction of natural enemies does not guarantee program success, and practitioners must be realistic about potential outcomes.

The results presented here suggest that control of individual kudzu plants in established kudzu patches could require more than 2 to 3 consecutive yr of severe damage. In a companion study of newly established kudzu plants, Frye et al. (2012) demonstrated that 75% leaf removal significantly reduced plant growth compared to a control. In the current study of established plants, however, only one of the treatments (50% complete vine removal) reduced aboveground biomass compared to undamaged controls, whereas plants in all other treatments compensated for biomass removal. Previous work with kudzu suggests that repeated removal of aboveground 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. Based on our results, we add that biomass removal of plants with smaller root crowns at the edge of kudzu's invasive range are more susceptible to damage-imposed mortality than plants with larger crowns. Therefore, we recommend that land managers consider implementing multiple control tactics when dealing with kudzu. This includes techniques to remove photosynthetic surfaces (mowing, burning, grazing, solarization), revegetation techniques to increase competition for soil resources and sunlight and to prevent seedling establishment, and systemic herbicide treatments targeting root structures.

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