ABSTRACT

KIDD, KATHLEEN ANN. Interaction of Kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) and Arthropods in North Carolina. (Under the direction of David B. Orr and Fred Gould).

This project was undertaken to better understand the ecology of kudzu growing in the United States, and to determine differences between plants from North Carolina and from the native range of this plant, in China. Specific goals of this project were to: 1) evaluate the development and feeding of a native herbivore, *Pseudoplusia includens* (Walker), the soybean looper, on kudzu and to determine if it could be used as a model herbivore for future studies; 2) determine the rate of seed herbivory at several locations in NC; 3) compare feeding on Chinese and US kudzu by generalist herbivores in the field and the model herbivore in the laboratory; 4) compare the growth and development of kudzu from Chinese and North Carolina seed sources; and 5) examine herbivore-induced volatile production and its effects on predation.

Larvae of *P. includens* were successfully reared on kudzu, but had higher mortality, longer development times with supernumerary molts, and lower pupal weights compared to larvae reared on soybean. Rearing history affected length of development time, but did not affect other measured parameters. Foliage consumption did not differ between treatments, and nutritional quality of soybean and kudzu did not differ. The stadia at which insects were transferred from artificial diet to either kudzu or soybean significantly influenced development time, number of stadia, foliage consumption, and pupal weights, with the fourth stadia being the optimum stage for transfer. When offered kudzu alone, females readily used it as an oviposition substrate, but when both kudzu and soybean were provided, more eggs

were deposited on soybean than on kudzu. Because kudzu is an acceptable host to *P. includens* this herbivore could be used in future studies as a model herbivore.

Insect herbivory on kudzu seeds was widespread across NC, with 80.9% of unprotected seeds damaged by insect feeding. Feeding by hemipterans caused the most damage statewide, and herbivory by a naturalized bruchid, *Borowecious ademptus,* was also widespread.

In the field, plants from China had less insect defoliation than plants from the US. In a laboratory bioassay, however, no evidence was found that plants from China were better defended from herbivory than plants from the US. No significant differences were found in preimaginal development times of *P. includens*, our model herbivore, or in the amount of foliage consumed. By comparison, *P. includens* fed soybean foliage developed in significantly less time and were larger than those fed on kudzu from either source.

Several growth parameters of kudzu from China and the US were compared in a field study. Vine length, vine dry weights, and the area (m^2) covered were greater for kudzu of Chinese origin than for plants of US origin. Although the ratio of root:vine dry weight for plants of Chinese origin was lower than for those of US origin, the ratio of vine dry weight to area covered by the plants was similar for plants from both sources. This suggests that Chinese plants directed more resources to vine growth than root growth, but of those resources directed to the vines, allocation was similar. Fiber content of the roots of Chinese plants was higher than that of US plants. These differences in vine growth and root fibers may be indicative of different varieties or biotypes of kudzu.

Herbivore-induced volatile production was examined in the field and the laboratory. Feeding by the herbivore *P. includens* resulted in an increase of visits by generalist predators at certain times of the year. Additional methods development is needed to produce conclusive data on volatile production by kudzu and its impact on predation.

INTERACTION OF KUDZU, *PUERARIA MONTANA* (LOUR.) MERR. VAR. *LOBATA* (WILLD.), AND ARTHROPODS IN NORTH CAROLINA

by

KATHLEEN A. KIDD

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Co-chair of Advisory Committee	Co-chair of Advisory Committee

BIOGRAPHY

Kathleen A. Kidd was born February 15, 1958, in West Palm Beach, Florida, to Joan and David Kidd. She was raised in Lake Worth, Florida, and attended public schools, graduating from Lake Worth High School in 1976.

Yearning for a change of seasons and landscape, she attended Tusculum College, a small school in the foothills of the Great Smoky Mountains of east Tennessee. There she became interested in the field of entomology, and upon graduation in 1980, moved to Raleigh to enroll at North Carolina State University. She received the Master of Science Degree in Entomology in 1983, and went to work as a technician in the department in 1984.

In 1992, she moved to a similar position at the North Carolina Department of Agriculture and Consumer Services. Upon promotion to the position of Biological Control Administrator in 1997, her supervisor suggested she work toward the Doctor of Philosophy degree. She enrolled in the degree program in the Department of Entomology at North Carolina State University in that year.

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Chapter 1

Comparative Feeding and Development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) on Kudzu and Soybean Foliage

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Abstract

Kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & Almeida, a close relative of soybean (*Glycine max* (L.) Merr.), is an adventive and widely distributed weed in the southeastern US. Conventional wisdom says that native arthropods do not feed heavily on kudzu, but recent evidence has indicated this is not the case. To better understand the interaction of kudzu and native insects, the biology of the soybean looper, *Pseudoplusia includens* (Walker), was compared on kudzu and soybean. Insects fed on kudzu had higher mortality, longer development times with supernumerary molts, and lower pupal weights than those fed on soybean. Rearing for up to three generations on either plant host had an effect on length of development time, but did not affect other measured parameters. Foliage consumption did not differ between treatments, and nutritional quality of soybean and kudzu did not differ. The instar at which insects were transferred from artificial diet to either kudzu or soybean significantly influenced development time, number of instars, foliage consumption, and pupal weights, with instar four being the optimum stage for transfer. In an oviposition test, females readily oviposited on kudzu in a no choice test, but when both kudzu and soybean were provided, more eggs were deposited on soybean than on kudzu. Our studies show that although kudzu is an acceptable host, soybean is a preferred and better quality host.

Keywords: kudzu, soybean, host preference, preimaginal development, fecundity, reproduction

Pueraria montana (Lour.) Merr. var. *lobata* (Willd.) Maesen & Almeida, (kudzu), is a common adventive weed in the southeastern US that belongs to the same tribe as soybean, *Glycine max* (L.) Merr. (family Fabaceae, subfamily Papilionatae (Lotoideae), tribe Phaseoleae) (Heywood 1971). Native to China, the vine is widely used in China and Japan for food and fiber (Duke 1981, Shurtleff and Aoyagi 1977). It was first introduced to the United States in 1876, at the Centennial Exposition in Philadelphia and promoted as an ornamental and for livestock feed. During the 1930s and 1940s, kudzu was recommended for erosion control, and in the six years from 1935 to 1940, the US Soil Conservation Service made available 73 million kudzu seedlings to landholders for erosion control (Tabor and Susott 1941).

Although quantitative data are lacking, anecdotal estimates suggest that over 7 million acres in the southeastern US are infested (Everest et al.1991), causing economic damage due to cost of control and lost production. Kudzu is widely acknowledged as a major weed pest of forests and rights of way in the Southeast. Biological control of kudzu is being explored, because other options may not be feasible. An important component of a weed biological control program is the study of the target weed’s biology and ecology as well as the population dynamics of its natural enemies in its area of origin. In addition, similar studies prior to the introduction of natural enemies from the area of origin are critical in the location where the plant has become a weed. Despite kudzu’s pervasiveness, almost nothing is known of its ecology and interactions with native arthropods in the USA.

To begin these studies, we looked for a model herbivore that could be used in both field and laboratory experiments. Few insects have been reported feeding on kudzu in the

US. The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, utilizes the plant as an overwintering host in central and south Florida (Buschman et al. 1977). In 1995, larvae of *A. gemmatalis*, *Pseudoplusia includens* (Walker) (soybean looper), and *Estigmene acraea* (Drury) (saltmarsh caterpillar) were observed feeding on kudzu near Union, SC (L. Barber and D. Orr, personal observation). We chose *P. includens* as a model herbivore in kudzu studies, because it is attacked by a common, native egg-larval parasitoid, *Copidosoma truncatellum* (Dalman) (Burleigh 1971, Deitz et al. 1976, Daigle et al.1990). This parasitoid allows hosts to complete larval development and spin cocoons then kills them just prior to pupation (Silvestri 1937). This would allow for the release of large numbers of insects into field plots without the concern of non-target impacts.

The soybean looper is a plusiine noctuid that feeds on numerous plants, but soybeans, cotton, and sweetpotatoes are its primary hosts (Hensley et al. 1964, Canerday and Arant 1967, Mitchell 1967, Herzog 1980). *Pseudoplusia includens* has been reported to feed on plants in 28 different families, including eight species of legumes (Herzog 1980). Martin et al. (1976) tested a number of crops including bush beans, field peas, peanuts, and soybeans and based on abundance of larvae on plants reported that soybeans were the preferred host followed by peanuts. Additional host plants included collards, bell peppers, and tomatoes, but *P. includens* did not oviposit or feed on bush beans and field peas. An annual legume, *Desmodium toruosum* (Swartz) de Candolle (Florida beggarweed) is also an acceptable host plant for *P. includens*, and laboratory studies demonstrated that it provides sufficient nutrition for the insect’s development (Beach and Todd 1988).

Pseudoplusia includens can be lab-reared successfully using an artificial diet (Shorey and Hale 1965, Burton 1969, Greene et al. 1976, Shour and Sparks 1981), an important factor for selecting it as a model insect for study. The development of the insect under various conditions has been described (Canerday and Arant 1967, Mitchell 1967, Reid and Greene 1973, King 1981, Shour and Sparks 1981, Trichilo and Mack 1989, Strand 1990). The purpose of our study was to compare the preimaginal development, foliage consumption, and reproduction of *P. includens* reared on kudzu compared to soybean in a laboratory study and to determine the optimum stadium to use for field studies.

Methods and Materials

Insect Colonies

Soybean looper larvae used in this study were originally collected from Open Grounds Farm, Carteret County, NC in October 1997 and maintained in a laboratory colony on an artificial diet (Burton 1969). Several additional subcolonies of *P. includens* were developed with the following rearing histories: 1) loopers from the laboratory colony; 2) loopers reared for one generation on soybean leaves (S-1); 3) loopers reared for one generation on kudzu leaves (K-1); 4) loopers reared for two generations on soybean leaves (S-2); and 5) loopers reared for two generations on kudzu leaves (K-2). For the S-1 and K-1 colonies, a new cohort was started each week with approximately 100 neonate larvae from the laboratory colony to provide a constant supply of insects. From 1 May to 3 July 1998, foliage was collected from greenhouse grown soybean (var. Brim) and kudzu plants. After 3 July, foliage was collected from plants grown in small plots on the NCSU campus. All leaves were rinsed with a dilute (0.035%) sodium hypochlorite solution then tap water to remove any entomopathogens prior to feeding (Beach et al. 1985) and placed in test tubes fitted with a single hole rubber stopper. Test tubes containing foliage were placed in 12 liter, square, polyethylene boxes (Rubbermaid, Twinsburg, OH) and held upright with wire supports. Foliage was added as needed, and pupae were removed and their numbers recorded as they developed. All pupae from a cohort were placed in a separate polyethylene box with sugar water for adult feeding and a leaf in a test tube as described above for oviposition. Initially, leaves of the same species on which the insects had developed were provided, but the quantity of eggs being laid on kudzu leaves was not satisfactory, so soybean leaves were provided in all oviposition boxes. Eggs were rinsed from the foliage with a dilute sodium hypochlorite solution (approx. 0.015%) and served as the foundation of the S-2 and K-2 subcolonies. All of these subcolonies were maintained in a growth chamber (Model 35VL, Percival, Boone, IA) at 25°C, 80% RH, and a photoperiod of 14:10 (L:D) (Beach and Todd 1988).

Feeding, Development, and Fecundity

To test the influence of plant species and rearing history on feeding, development, and fecundity, *P. includens* reared 0, 1, or 2 generations on either kudzu or soybean were compared in a laboratory experiment. The experiment was a 2 by 5 factorial design. Treatments were kudzu or soybean, and the factors were the five rearing histories described above (K-1, K-2, S-1, S-2, or Diet). The experiment was conducted twice with 25 replications in trial 1 (18 July – 11 August 1998) and 15 in trial 2 (27 August – 19 September 1998).

The feeding arena used in this study was an inverted 150 by 15 mm plastic petri dish, with moistened filter paper lining the lid. Neonate larvae from each subcolony were randomly assigned to individual dishes within a treatment group and provided with an individual leaflet from the first fully expanded leaf from soybean plants and fully expanded young leaves from kudzu vines. Foliage was collected and treated as described for insect colonies.

All dishes were held at 25°C, 80 % RH, and 14:10 (L:D) photoperiod in an environmental chamber (Model 35VL, Percival, Boone, IA) (Boldt et al. 1975, Richter and Fuxa 1984, Beach and Todd 1988). To control error due to variability of temperature, light or humidity within the chamber, dishes were arranged in a randomized complete block design, consisting of 5 or 3 blocks (trials 1 and 2, respectively) with 5 dishes of each treatment combination per block. Leaflets were changed every 48 h or as needed.

Larval development was recorded as the length of time spent in each stadium and total development time. Throughout the study, dishes were examined twice daily at 1 h after lights on and 2 h before lights out, and the day of molt to a new instar was recorded. Shour and Sparks (1981) found no differences in weights of *P. includens* larvae reared on artificial media until the fourth larval instar, regardless of the number of instars required to complete development. Therefore, beginning in the fourth instar, larvae were weighed in the premolt stage to the nearest 0.1 mg.

When the larvae reached the fourth or fifth instar, foliage consumption was estimated by measuring individual leaflets with an area meter (Model LI-3100 Li-Cor, Lincoln, NE). During the first trial we found that feeding by fourth instar larvae on kudzu was not measurable with the area meter, therefore measurements were initiated with the fifth instar in the second trial. To compensate for different leaflet thickness, feeding rate was estimated as the consumption of dry matter. At three points during each trial, 10 leaflets of each plant species were measured in the area meter and then weighed to the nearest 0.1 mg with an analytical balance (Model M220D, Denver Instrument Co., Arvada, CO). Leaves were dried in an oven at 45°C for at least 72 h, then weighed, and the percentage of dry matter was converted to the weight (mg) of dry matter per leaf area (cm^2) (Beach and Todd 1986, 1988). The amount of leaf area consumed in the experiment was converted to mg dry matter consumed using the average dry matter value.

Ten leaflets of each species were used to determine the amount of shrinkage due to water loss during the feeding period. Measured leaves were placed in individual petri dishes identical to the feeding dishes, randomly placed in the incubator, and held under the same conditions. Leaflets were set up every 1.5 to 3 d throughout the test period when leaf area was measured. Approximately 4 liters of fresh foliage were collected from the NCSU campus plots and submitted to the Forage Testing Laboratory of the North Carolina Department of Agriculture and Consumer Services for standard forage analysis.

Day of pupation was recorded, and pupae were weighed within 24 h. Sex of the pupae was determined, and up to 10 females (depending on how many females completed development) from each treatment group were randomly selected for the fecundity study. To measure fecundity, individual females from each treatment and two males from the laboratory colony were placed in paper cartons (0.5 liter) (Sweet Paper Co., Raleigh, NC) lined with waxed paper 6 d after pupation. Cartons were placed back in the incubator where rearing dishes were held, maintaining the same randomization. As a control, 10 randomly selected females from the laboratory colony that eclosed approximately midway between the soybean-fed and kudzu-fed insects were placed in cartons as above. Control females were placed in the incubator in the position previously occupied by the dishes holding leaves measured for shrinkage. All cartons were supplied with a 16% sucrose solution in 30 ml plastic cups (Polar Plastics, Mooresville, NC) for the moths and an excised soybean leaflet in an Aquapic™ (Syndicate Sales, Kokomo, IN). Tops of the cartons were covered with cheesecloth (50 grade, American Fiber and Finishing, Burlington, MA) held in place by the rim of the lid. Three days after the leaflets were introduced, and every 3 d until the female died, the leaflet, sugar water cup, waxed paper, and cheesecloth were changed. All materials from an oviposition carton were bagged and frozen at –17C until the eggs were counted.

Diet Switching Study

The purpose of this study was to determine the optimum stadium for use in field studies. The experiment was designed as a 2 by 5 factorial, with treatments being kudzu or soybean and the factors being developmental stage (instars 1-5) at which larvae were moved from artificial diet to leaves. Each treatment by factor group consisted of 24 replications; 24 larvae served as controls, and the larvae remained on diet throughout their development. Neonate larvae from the laboratory colony were randomly assigned to a treatment by factor group and fed artificial diet until reaching the assigned stage to be placed on foliage. Artificial diet prepared in 30 ml cups was sliced into disks approximately 5 mm thick and pressed gently onto the inner surface of the bottom half of a 150 by 15 mm plastic petri dish. When larvae were fed leaflets, petri dish lids were lined with moistened filter paper, but when diet was provided, no filter paper was used. Leaflets were field collected from plots on the NCSU campus and treated as in the feeding study. Soybeans were mistakenly fertilized with an unknown quantity of controlled-release fertilizer (Osmocote 14-14-14, Scotts Co, Columbus, OH) after planting in 1999, but none was applied to kudzu plantings. Dishes were arranged in a completely randomized design in a rearing room at 24.5°C (± 1.5°C), 70% RH, and14:10 L:D photoperiod. Larvae were examined once a day, and the day of molting to a new instar was recorded. The number of instars required to complete development and day of pupation were recorded. Within 24 h of pupation, sex was determined, and pupae were weighed to the nearest 0.1 mg. When larvae reached the fifth instar, foliar feeding was measured as dry matter (mg) consumed as in the feeding study. Leaf shrinkage or expansion was also considered as in the feeding study.

Oviposition Choice Test

To test the influence of host plant on oviposition in a laboratory experiment, *P. includens* reared on artificial media were offered three foliage treatments: 1) 1 soybean leaf, 2) 1 kudzu leaf, or 3) 1 kudzu and 1 soybean leaf. Testing arenas were cylindrical cages, 30.5 cm in diameter and 30.5 cm high, constructed of aluminum window screen (7 mesh/cm) with a cardboard bottom and screen top. Sugar water (16%) was supplied for the moths.

Female pupae were weighed within 24 h of pupation and held individually in 30 ml cups until eclosion. Upon eclosion, females of the same age were placed individually into arenas with two males that eclosed on the same or next day. Arenas were arranged in a randomized complete block design with selective placement of treatments on a metal shelving unit with 3 shelves. Each shelf was divided into 2 blocks (front and back halves) to control error due to variability in lighting or air circulation. The experiment was conducted in a rearing room maintained at 24.5°C (±1.5°C), 70% RH, and 14:10 (L:D) photoperiod, with two small nightlights to simulate moonlight.

Three days after female eclosion, foliage for oviposition was introduced. The first fully expanded leaves from the terminals of either kudzu vines or soybean plants were collected and treated as in the feeding study. Leaf area was measured with the area meter and only those leaves with an area of approximately 240-280 cm^2 were used. Leaves were placed individually in water-filled test tubes fitted with rubber stoppers held upright in the arenas and monitored every 24 h until oviposition started. When oviposition began, leaves were discarded, and replaced with fresh leaves. After 24 h, these leaves were removed, frozen at –17C and replaced with a second set of leaves that was also removed and frozen after 24 h. Eggs on these leaves were later counted, and numbers for both days were summed for each female.

Data analysis

To achieve homogeneity of variance, pupal weight, fecundity and oviposition data were square root transformed, survival data were arcsine transformed, and data for the fourth and fifth instar larval weights were log transformed. Only data for individuals that survived to pupation were analyzed. All data were analyzed using the general linear model procedure (PROC GLM, SAS Institute 1996), and means were separated with the least significant difference (LSD, SAS Institute 1996).

Results

Feeding, Development, and Fecundity

Survival to pupation of *P. includens* larvae feeding on kudzu was significantly lower than that of larvae feeding on soybeans (61.5% vs. 94.5%) ($F = 34.3$; df = 1, 9; $P \leq 0.001$); rearing history of larvae had no significant effect on survival on either of the plants ($F = 0.8$; df = 4,9; $P \geq 0.05$) (Table 1). Feeding on kudzu resulted in significantly increased development times (19.2 vs. 13.2 days) ($F = 759.4$; df = 1, 60; $P \leq 0.001$) and number of instars (6.8 vs. 5.3) ($F = 348.1$; df = 1, 9; $P \leq 0.001$). Rearing history had a significant effect on development time ($F = 3.0$; df = 4, 60; $P \leq 0.001$). Insects reared on either kind of foliage for two generations prior to the study tended to have longer development times than other larvae feeding on the same plant, but differences were not always statistically significant.

Larvae that had fed on soybean were twice as large as those fed on kudzu by the fourth instar ($F = 269.3$; df = 1, 9; $P \leq 0.001$). Significant differences due to host plant ($F = 276.9$; df = 1, 0; $P \leq 0.001$) and sex ($F = 8.9$; df = 1, 233; $P \leq 0.001$) were also found in the weights of pre-molt fifth instar larvae (data not shown). Pupae from the kudzu treatments were significantly smaller (average weight 173.4 mg) than soybean-reared *P. includens* (231.6 mg) ($F = 126.6$; df = 1, 9; $P \leq 0.001$). Male pupae were significantly larger than females (216.9 ± 40.9 mg vs. 201.0 ± 42.8 mg) ($F = 23.7$; df = 1, 233; $P \leq 0.001$).

No significant differences in the fecundity of females due to plant ($F = 1.7$; df = 1, 10; $P \geq 0.05$) or rearing history ($F = 1.3$; df = 4, 10; $P \geq 0.05$) were found. A relationship between pupal weight and the number of eggs was found ($F = 6.1$; df = 1, 102; $P \leq 0.001$).

However, pupal weight was affected by plant, and it can not be considered a true covariate with fecundity.

No significant differences in the total amount of foliage consumed were found regardless of plant type ($F = 0.7$; df = 1, 9; $P \geq 0.05$) or feeding history ($F = 0.4$; df = 4, 9; $P \geq 0.05$). Forage analysis showed that the two plants have similar crude protein content (23.7 and 24.7% for kudzu and soybean, respectively), and dry matter content was slightly higher for soybean (25%) compared to kudzu (22.5%).

Diet Switching Study

Larvae fed kudzu had significantly longer development times ($F = 47.2$; df = 1, 228; $P \leq 0.001$; $F = 31.1$) and lower pupal weights ($F = 236.9$; df = 1, 228; $P \leq 0.001$) than larvae fed soybeans or artificial diet (Table 2). Those switched in the first through fourth instars required more molts to complete development than other larvae ($F = 31.1$; df = 1, 228; $P \leq 0.001$). The stadium at which larvae were switched also had an effect, although a pattern is clearer for the larvae fed on kudzu ($F = 11.2$; df = 4, 228; $P \leq 0.001$). The earlier in their development that larvae were switched to kudzu, the longer their development time and the greater the number of molts. Larvae switched to kudzu for the fourth instar developed in a significantly shorter time (LSD; $P \leq 0.001$), but consumed a similar amount of food to those switched in earlier instars (LSD; $P \geq 0.05$). This trend was not seen for larvae switched to soybean at this stage. *Pseudoplusia includens* switched from artificial diet to kudzu for the fifth instar had a significantly shorter development time and fewer instars than other kudzu feeders (LSD; $P \leq 0.001$), but lower pupal weights than all other treatments (LSD; $P \leq 0.001$). In contrast to the feeding study presented above, significant differences in food consumption were found based on plant ($F = 111.6$; df = 1, 206; $P \leq 0.001$), stadium at which larvae were switched ($F = 18.9$; df = 4, 206; $P \leq 0.001$), the interaction of plant and stadium ($F = 8.6$; df = 4, 206; $P \leq 0.001$), and sex ($F = 8.8$; df = 1, 206; $P \leq 0.001$). Larvae switched to kudzu in the first through fourth instars consumed similar amounts of dry matter (LSD, $P \geq 0.05$). Larvae switched to kudzu in the fifth instar consumed similar amounts of dry matter as those switched to soybean at all stadia but the second.

Oviposition Choice Test

When presented soybean and kudzu in choice and no-choice tests, *P. includens* laid significantly more eggs on soybean leaves in either treatment than on kudzu in either treatment ($F = 91.64$; df = 1, 68; $P \leq 0.001$) (Table 3). The fewest eggs were laid on kudzu in the choice treatment; significantly more eggs were laid on kudzu in the no-choice treatment. Mean leaf areas ranged from 255.0-267.3 cm^2. Despite this narrow range, kudzu leaves were significantly larger than soybean leaves ($F = 7.74$; df = 3,80; $P \leq 0.001$; $F = 9.8$; df = 1, 72; $P \leq 0.001$). Weights of pupae were not significantly different by treatment ($F = 1.4$; df = 2, 79; $P \geq 0.05$).

Discussion

Results of this study show that kudzu is an acceptable host plant for *P. includens* development and reproduction. However, larvae fed kudzu showed increased mortality, longer development times, supernumerary molts, and lower pupal weights compared to larvae fed soybean. The effect of feeding on kudzu is comparable to that seen when *P. includens* develops on a mildly resistant soybean genotype or a less preferred host (Beach et al. 1985, Beach and Todd 1986, Beach and Todd 1988). Beach and Todd (1988) demonstrated that mortality of *P. includens* on a susceptible soybean variety was 4% compared to 16% for larvae fed on Florida beggarweed (*Desmodium toruosum*) and a resistant soybean line (GatIR 81-296). *Pseudoplusia includens* fed on resistant soybeans required approximately 2 d longer to pupate and had lower pupal weights than *P. includens* feeding on a susceptible variety (Beach et al. 1985, Beach and Todd, 1986). Development time also increased for *P. includens* fed cotton compared to larvae fed soybeans (Wier and Boethel 1995a). Additional studies of *P. includens* development have shown that its growth rate is affected by a number of factors including leaf position, plant age, and wounding on a resistant line of soybeans (Reynolds and Smith 1985). Larval development time and mortality were inversely affected by the level of nitrogen fertilizer (Wier and Boethel 1995b).

Size differences were observed at the fourth instar; larvae fed kudzu were smaller than those fed soybean. The number of instars required for pupation is associated with a minimum weight at the end of the penultimate instar (Strand 1990). Larvae feeding on kudzu did not attain the body size to complete development after five instars; therefore they required more molts. The range of mean pupal weights in our feeding study regardless of treatment (165.7 – 234.0 mg) was within the range of pupal weights reported in other studies (Mitchell 1967, Reid and Greene 1973 Jensen et al. 1974, Felland and Pitre 1991, Wier and Boethel 1995b). Higher values have also been reported (Canerday and Arant 1967, Kogan and Cope 1974).

The mean soybean dry matter consumed in the feeding and diet switching studies falls within the range of soybean consumption found in previous studies (Reid and Greene 1973, Kogan and Cope 1974, Beach and Todd 1988). In our development study, the amount of kudzu consumed was not significantly different from the amount of soybean consumed. In contrast, larvae switched from diet to kudzu foliage in the first through fourth instars consumed significantly more dry matter than those feeding on soybeans. One possible explanation for these observations may be the lack of fertilization for kudzu plots compared to soybean. No fertilizer was applied to kudzu plots, while fertilizer was applied to soybean plots. Nitrogen fertilization affects the nutritional makeup of plants, and the ability to convert plant N impacts an insect's ability to grow and reproduce (Scriber 1984). Beach and Todd (1988) found the greatest amount of feeding occurred on a susceptible soybean variety and less on a weed and resistant soybeans. Wier and Boethel (1995a) reported *P. includens* consumed significantly less cotton foliage than soybean foliage, an indication that cotton is less suitable as a host plant than soybeans.

Egg production in our study ranged from 766.8 to 1441.4 eggs per female and was high compared to other studies (Mitchell 1967, Jensen et al. 1974, Beach et al. 1985). Mitchell (1967) reported an average number of 240.2 eggs deposited per female, and Jensen et al. (1974) reported egg numbers between 425.9 and 671.3 eggs/female from a series of experiments on adult nutrition. In a comparison of susceptible and resistant lines of soybean, *P. includens* that developed on a susceptible line (Ransom) deposited an average of 340 eggs/female, and significantly fewer eggs were laid on resistant varieties (Beach et al. 1985).

In the laboratory, kudzu is an acceptable, but less suitable and preferred host plant compared to soybean for *P. includens.* Interestingly, Thornton (2000) demonstrated that foliage feeding in the field by native and naturalized insects resulted in seasonal levels of defoliation in kudzu identical to that in soybean.

Both soybean and kudzu originated in Asia and appear to harbor the same communities of many generalist herbivores (Thornton 2000). Before any biological control agents are introduced to the United States from China, we feel it is important to develop at least a basic understanding of the ecology of kudzu and its associated arthropods in both countries. This approach has been recommended by McClay (1995) as one way to improve the overall success of classical biological control programs against weeds. The information collected in this study will enable us to use *P. includens* as a model foliar-feeding insect for comparisons between kudzu populations from the United States and China and provide baseline information to study the interaction of native predators and parasitoids with foliage-feeding insects on kudzu in the field.

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References Cited

Beach, R.M. and J.W. Todd. 1986. Foliage consumption and larval development of parasitized and unparasitized soybean looper, *Pseudoplusia includens* [Lep.: Noctuidae], reared on a resistant soybean genotype and effects on an associated parasitoid, *Copidosoma truncatellum* [Hym.: Encyrtidae]. Entomophaga. 31: 237-242.

Beach, R.M. and J.W. Todd. 1988. Comparative consumption and utilization of Florida beggarweed and two soybean genotypes by *Pseudoplusia includens* larvae (Lepidoptera: Noctuidae). J. Entomol. Sci. 23: 97-104.

Beach, R.M., J.W. Todd, and S.H. Baker. 1985. Antibiosis of four insect-resistant soybean genotypes to the soybean looper (Lepidoptera: Noctuidae). Environ. Entomol. 14: 531-534.

Boldt, P.E., K.D. Biever, and C.M. Ignoffo. 1975. Lepidopteran pests of soybeans: consumption of soybean foliage and pods and development time. J.Econ. Entomol. 68: 480-482.

Burleigh, J.G. 1971. Parasites reared from the soybean looper in Louisiana 1968-69. J. Econ. Entomol. 64: 1550-1551.

Burton, R.L. 1969. Mass rearing of the corn earworm in the laboratory. US Dept. Agric. Res. Serv. [Rep.] ARS-33-134.

Buschman, L.L., W.H. Whitcomb, T.M. Neal, and D.L. Mays. 1977. Winter survival and hosts of the velvetbean caterpillar in Florida. Fla. Entomol. 60: 267-273.

Canerday, T.D. and F.S. Arant. 1967. Biology of *Pseudoplusia includens* and notes on biology of *Trichoplusia ni*, *Rachiplusia ou*, and *Autographa biloba*. J. Econ. Entomol. 60: 870-871.

Daigle, C.J., D.J. Boethel, and J.R. Fuxa. 1990. Parasitoids and pathogens of soybean looper and velvetbean caterpillar (Lepidoptera: Noctuidae) in soybeans in Louisiana. Environ. Entomol. 19: 746-752.

Deitz, L.L., J.W. Van Duyn, J.R. Bradley, Jr., R.L. Rabb, W.M. Brooks, and R.E. Stinner. 1976. A guide to the identification and biology of soybean arthropods in North Carolina. Tech. Bull. No. 238. NC Agr. Res. Ser. 264 pp.

Duke, J.A. 1981. Handbook of Legumes of World Economic Importance. Plenum Press. New York.

Everest, J.W., J.H. Miller, D.M. Ball, M.G. Patterson. 1991. Kudzu in Alabama. History, uses, and control. Circ. ANR-65.8 pp.

Felland, C.M. and H.N. Pitre. 1991. Survivorship and development of noctuid larvae (Lepidoptera: Noctuidae) on irrigated and dryland soybean. Environ. Entomol. 20:1315-1321.

Greene, G.L., N.C. Leppla, and W.A. Dickerson. 1976. Velvetbean caterpillar: a rearing procedure and artificial medium. J. Econ. Entomol. 69:487-488.

Hensley, S.D., L.D. Newsom, and J. Chapin. 1964. Observations on the looper complex of the noctuid subfamily Plusiinae. J. Econ. Entomol. 57: 1006-1007.

Herzog, D.C. 1980. Sampling soybean looper on soybean. *In*: Sampling Methods in Soybean Entomology. M. Kogan and D.C. Herzog, [eds], pp. 141-168. Springer–Verlag. New York.

Heywood, V.H. 1971. The leguminosae – a systematic purview. *In*: Chemotaxonomy of the Leguminosae. Harborne, J.B., D. Boulter, and B.L. Turner, [eds], pp 1-29. Academic Press. London.

Jensen, R.L., L.D. Newsom, and J. Gibbens. 1974. The soybean looper: Effects of adult nutrition on oviposition, mating frequency, and longevity. J. Econ. Entomol. 67: 467-470.

King, E.W. 1981. Rates of feeding by four lepidopterous defoliators of soybeans. J. GA Entomol. Soc. 16: 283-288.

Kogan, M. and D. Cope. 1974. Feeding and nutrition of insects associated with soybeans. 3. Food intake, utilization, and growth in the soybean looper, *Pseudoplusia includens*. Ann. Entomol. Soc. Amer. 67: 66-72.

Martin, P.B., P.D Lingren, and G.L. Greene. 1976. Relative abundance and host preferences of cabbage looper, soybean looper, tobacco budworm, and corn earworm on crops grown in Northern Florida. Environ. Entomol. 5: 878-882.

McClay, A. S. 1995. Beyond "Before-and-After:" Experimental design and evaluation in classical weed biological control, pp 213-219. *In* E.S. Delfosse and R.R. Scott [eds.], Proceedings, VIII International Symposium Biological Control of Weeds, 2-7 February 1992, Canterbury, NZ. CSIRO Publishing, Melbourne, AU.

Mitchell, E.R. 1967. Life history of *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae). J. GA Entomol. Soc. 2:53-57.

Reid, J.C. and G.L. Greene. 1973. The soybean looper: pupal weight, development time, and consumption of soybean foliage. Fla. Entomol. 56: 203-206.

Reynolds, G.W. and C.M. Smith. 1985. Effects of leaf position, leaf wounding, and plant age of two soybean genotypes on soybean looper (Lepidoptera: Noctuidae) growth. Environ. Entomol. 14: 475-478.

Richter, A.R. and J.R. Fuxa. 1984. Preference of five species of noctuidae for feeding-stimulant adjuvants. J. GA. Entomol. Soc. 19: 383-387.

SAS Institute. 1996. SAS User's manual, version 6. SAS Institute, Cary, NC.

Scriber, J. M. 1984. Nitrogen nutrition of plant and insect invasion, pp 441-460. *In* R.D. Hauck [ed], Nitrogen in crop production. ASA-CSSA-SSSA, Madison, WI.

Shorey, H.H. and R.L. Hale. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. J. Econ. Entomol. 58: 522-524.

Shour, M.H. and Sparks, T.C. 1981. Biology of the soybean looper, *Psuedoplusia includens*: Characterization of last-stage larvae. Ann. Entomol. Soc. Am. 74: 531-535.

Shurtleff, W. and A. Aoyagi. 1977. The book of kudzu. A culinary and healing guide. Autumn Press. Brookline, MA.

Silvestri, F. 1937. Insect polyembryony and its general biological aspects. Bull. Mus. Comp. Zool. Harvard Coll. 81: 469-498.

Strand, M.R. 1990. Characterization of larval development in *Pseudoplusia includens* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 83: 538-544.

Tabor, P. and A.W. Susott. 1941. Zero to thirty million mile-a-minute seedlings. Soil Conservation. 7: 61-65.

Thornton, M.R. 2000. Native and naturalized arthropod fauna of kudzu (*Pueraria lobata* Willd.) in North Carolina. MS thesis, North Carolina State University. (in prep.)

Trichilo, P.J. and T.P. Mack. 1989. Soybean leaf consumption by the soybean looper (Lepidoptera: Noctuidae) as a function of temperature, instar, and larval weight. J. Econ. Entomol. 82: 633-638.

Wier, A.T. and D.J. Boethel. 1995a. Foliage consumption and larval development of three noctuid pests on soybean and cotton. J. Entomol. Sci. 30: 359-361.

Wier, A.T. and D.J. Boethel. 1995b. Feeding, growth, and survival of soybean looper (Lepidoptera: Noctuidae) in response to nitrogen fertilization of nonnodulating soybean. Environ. Entomol. 24: 326-331

Table 1. Development, dry weight of foliage consumed and total number of eggs laid by *P. includens* with various feeding histories on kudzu and soybean foliage in the laboratory. (Mean ± SD).

Plant[a]	History[b]	n	% Survival	No.days to pupation	No. instars	4^{th} Instar wt (mg)[c]	Pupal wt (mg)	Consumption (mg dry wt)	n( )	Eggs/female
Kudzu	Diet	20	50.0 ± 33.8b	18.9 ± 1.3cd	6.8 ± 0.6b	6.8 ± 1.9b	169.4 ± 2.8b	287.0 ± 147.3a	6	924.7 ± 567.4a
	K-1	25	62.5 ± 22.5b	18.7 ± 1.4c	6.7 ± 0.5b	6.1 ± 2.0b	165.7 ± 3.6b	306.6 ± 112.3a	9	766.8 ± 413.8a
	K-2	30	75.0 ± 20.7b	19.4 ± 2.3de	6.8 ± 0.7b	6.7 ± 2.0b	178.4 ± 3.8b	341.0 ± 130.2a	14	808.1 ± 540.1a
	S-1	23	57.5 ± 19.8b	19.6 ± 1.7e	6.9 ± 0.5b	6.9 ± 1.7b	176.4 ± 3.3b	303.0 ± 133.9a	7	871.3 ± 474.3a
	S-2	25	62.5 ± 32.8b	19.2 ± 1.8cde	6.9 ± 0.6b	6.8 ± 2.4b	175.4 ± 4.3b	310.4 ± 134.6a	11	894.3 ± 701.3a
	Total n/Mean	123	61.5 ± 26.6	19.2 ± 1.8	6.8 ± 0.6	6.7 ± 2.0	173.4 ± 3.6	311.4 ± 131.8	47	844.7 ± 535.4
Soybean	Diet	38	95.0 ± 9.3a	12.7 ± 0.8a	5.2 ± 0.4a	14.6 ± 4.5a	231.7 ± 3.5a	274.5 ± 73.5a	17	878.2 ± 680.0a
	K-1	38	95.0 ± 9.3a	12.9 ± 0.8a	5.2 ± 0.4a	12.8 ± 3.2a	230.4 ± 2.3a	297.3 ± 87.4a	18	1109.7 ± 620.7a
	K-2	38	95.0 ± 9.3a	13.6 ± 1.5b	5.3 ± 0.4a	14.6 ± 3.8a	232.3 ± 3.2a	304.5 ± 88.0a	15	943.6 ± 613.2a
	S-1	38	95.0 ± 9.3a	13.3 ± 0.7ab	5.2 ± 0.4a	13.7 ± 3.7a	228.8 ± 2.8a	270.0 ± 70.8a	17	1106.4 ± 423.1a
	S-2	37	92.5 ± 10.4a	13.6 ± 1.1b	5.4 ± 0.5a	12.7 ± 4.9a	234.0 ± 2.2a	300.9 ± 84.5a	16	1447.4 ± 595.1a
	Total n/ Mean	189	94.5 ± 9.1	13.2 ± 1.1	5.3 ± 0.5	13.7 ± 4.1	231.6 ± 2.9	284.2 ± 81.2	83	1096.7 ± 610.1
Colony									17	1147.3 ± 807.0a

Values within a column followed by the same letter are not significantly different. (P>0.05, LSD).

[a]Plant: species being fed upon in the test, kudzu or soybean.

[b]History: food previous generation(s) reared on: Diet = insects from lab colony; K-1, S-1 = 1 generation reared on kudzu or soybean foliage; K-2, S-2 = 2 generations reared on kudzu or soybean foliage.

[c]Purged larvae were weighed within 12 h of molting to the 4^{th} instar.

Table 2. Development of *P. includens* switched from artificial diet to kudzu and soybean foliage at different stages in the laboratory. (Mean ± SD).

Plant[a]	Stage[b]	n	Days to pupation	Wt of pupae (mg)	No. instars	Consumption (mg dry wt)
Kudzu	1	21	20.2 ± 1.9a	200.8 ± 30.8e	7.0 ± 0.6a	493.3 ± 112.4a
	2	22	20.4 ± 1.4a	203.1 ± 22.4e	7.3 ± 0.5a	481.7 ± 81.2a
	3	22	19.9 ± 2.1a	186.4 ± 38.8e	6.7 ± 0.5b	468.1 ± 107.2a
	4	22	18.5 ± 1.3b	193.9 ± 28.1e	6.3 ± 0.5b	471.8 ± 107.3a
	5	22	16.2 ± 1.9c	152.3 ± 50.6f	5.6 ± 0.8de	254.0 ± 133.9c
Soybean	1	24	13.0 ± 1.1f	248.9 ± 32.9bcd	5.1 ± 0.3f	276.8 ± 57.3bc
	2	23	14.4 ± 1.2d	269.1 ± 27.0a	5.8 ± 0.5d	313.8 ± 47.0b
	3	24	13.5 ± 1.4ef	253.3 ± 20.4abc	5.6 ± 0.5ef	289.1 ± 44.1bc
	4	23	13.9 ± 0.6de	262.2 ± 27.2ab	5.5 ± 0.5e	307.9 ± 64.3bc
	5	23	14.4 ± 1.3d	232.8 ± 51.1d	5.4 ± 0.5ef	256.8 ± 68.1c
Diet	–	20	14.2 ± 1.3de	240.2 ± 35.4cd	5.4 ± 0.5ef	–

Values within a column followed by the same letter are not significantly different. (P>0.05, LSD).

[a]Plant: species being fed upon in the test, kudzu or soybean, or artificial diet.

[b]Stage: instar at which larvae were switched from artificial diet to foliage.

Table 3. Number of *P. includens* eggs laid on kudzu and soybean in oviposition choice and no choice tests in the laboratory (Mean ± SD).

	Oviposition substrate	n	Wt of pupae (mg)	Leaf area (cm^2)	Eggs/ Female
No Choice	Kudzu	28	215.4 ± 28.9a	264.8 ± 10.4ab	325.7 ± 202.9b
	Soybean	26	221.5 ± 34.7a	259.6 ± 11.3bc	570.9 ± 228.7a
Choice	Kudzu	28	224.6 ± 29.9a	267.3 ± 10.9a	44.0 ± 72.8c
	Soybean	28	224.8 ± 29.9a	255.0 ± 9.1c	468.5 ± 224.3a

Values followed by the same letter are not significantly different (P>0.05, LSD).

Chapter 2

Herbivory on Kudzu (*Pueraria montana* var. *lobata)*

Seeds in North Carolina

Abstract

Kudzu (*Pueraria montana* var. *lobata*) seeds from 23 locations, representing the 3 major geographic areas of North Carolina were examined in 1999 to determine the extent of insect feeding damage. Seeds were either protected with exclusion cages from an early stage of development or left unprotected. Damage to unprotected seeds was high across the state, with hemipteran damage being the most common. A naturalized bruchid that develops within seeds was also widespread, and in two counties, bruchid damage exceeded hemipteran damage. Only 19.1 % of non-caged seeds were free of insect damage, compared to 90.9% of caged seeds.

Kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & Almeida, a member of the Fabaceae, is an herbaceous or semi-woody vine (Mitich 2000). The plant is propagated either vegetatively using crowns or cuttings or by seed (Duke 1981). Kudzu does not flower and produce seed until it is about three years old, and typically only vines that are growing vertically on some support such as a tree, structure, or even on the side of a gully produce flowers (Tabor and Susott 1941, Tabor 1942, Dabadghao 1949). Numerous factors affect the quantity and quality of seed produced, including rainfall, pollination and insect herbivory (Tabor and Susott 1941, Abramavitz 1983).

Feeding by different insects results in characteristic damage. Hemipterans and a naturalized bruchid caused the most common damage found in Wake Co., NC (M.R. Thornton, unpublished data). Seeds with hemipteran damage early in their development are shriveled and darkened with a puncture wound from the insect stylet. Later feeding by hemipterans results in a puncture wound without the shriveled appearance, and discoloration only around the wound. A variety of hemipterans may attack the seeds of kudzu, among them the green stink bug, *Acrosternum hilare* (Say) (Hemiptera: Pentatomidae). Bruchid feeding results from *Borowiecius ademptus* (Sharp) (Coleoptera: Bruchidae) a naturalized insect of Asian origin. Seeds damaged by this insect are misshapen (lumpy), and have an oviposition wound that is larger than the hemipteran feeding puncture. Seeds may also have a round emergence hole left by *B. ademptus*. The bruchid was described from Japan in 1886, and was collected in NC in 1943 (Bottimer 1961). It was found in a kudzu seed nursery in Alabama in 1941, most likely imported with seed from Japan (Bottimer 1961).

Previous studies in Wake Co, NC, indicated that high levels of insect feeding occur on unprotected seeds, with 89-98% of seeds damaged (M.R. Thornton, unpublished data). To determine if this level of seed damage was typical, kudzu seeds collected from 23 locations across North Carolina were studied using cage exclusion techniques.

Methods and Materials

Kudzu seeds for use in a growth study (Chapter 4, this dissertation) were collected from 23 locations in the three major geographic regions (coastal plain, piedmont, and mountains) of North Carolina. Multiple sites in each region were targeted, and if more than one site within a county was sampled, an effort was made to select sites at least five miles apart. Kudzu stands were selected based on accessibility and the presence of flowers at the desirable stage. To protect the developing seeds, racemes were caged with organdy cloth sewn into bags (14 cm X 25 cm) with a 7 cm opening. Older pods and the tip of the raceme that had not flowered were removed, retaining flowers that showed the beginning of fruit formation and very young pods. The opening was closed around the raceme with plastic coated wire (Dispens-O-Wire®, Anchor Wire Corp., Goodlettsville, TN) and labeled with aluminum tags (Ben Meadows Co, Canton, GA). Racemes were caged between 16 August and 9 September 1999 and collected when the seeds had matured, about 60 days later. At most sites, when cages and seed were collected, nearby uncaged seed pods were collected to compare herbivory.

Pods were stored at 7.2°C until processed in the laboratory. The seeds were removed from the pods, counted, then placed in small envelopes, by pod. Seeds were later examined under a dissection microscope (Wild MZ8, Leica Microsystems, Inc., Bannockburn, IL) for insect damage. Damage was classified as: 1) none, 2) early hemipteran, 3) late hemipteran, 4) bruchid and 5) other, caused by unknown insects or agents.

All data were analyzed using the general linear model procedure (PROC GLM, SAS Institute 2001). A two-way ANOVA with factors “location” and “cage” was carried out, and means were separated using the least significant difference (LSD).

Results and Discussion

Seeds were collected from 23 locations in 19 counties across NC (Table 1) (Figure 1). Seed production ranged from 1.2 to 9.1 seeds per pod with a mean of 3.5 seeds overall (Table 2). Tsugawa (1986) reviewed several papers on kudzu under cultivation and reported yields of 4.5 – 4.8 seeds/pod. At many locations, especially in the mountain counties, vines flowered, but no fruit formed. Both location and cage had a significant effect on the number of seeds produced ($F = 31.64$; df = 24, 780; $P < 0.0001$; $F = 4.89$; df = 1; 780; $P < 0.05$). Low seed production throughout the state may have been due to drought conditions and the lowest numbers of seeds were found in the mountain counties. The best seed set in kudzu occurs in years with above average rainfall (Tabor and Susott 1941), and by August 1999 all of NC was experiencing dry conditions with the western half of the state in drought status (Drought Monitor 1999).

Significant differences in the percent of seeds damaged were found by location and presence of a cage ($F = 21.13$; df = 24, 778; P 0.0001; $F = 837.33$; df = 1, 778; P 0.0001) (Table 2). Bruchid damage was widespread and location and cage were significant factors ($F = 7.5$; df = 24, 780; P 0.0001; $F = 117.88$; df = 1,780; P 0.0001). No bruchid damaged seeds were found in the westernmost counties, but this may have been due to the small collections of unprotected pods from these counties. Little bruchid damage was found in caged seeds from any county, while over half of the unprotected seeds collected at the Granville 2, Johnston, and Rockingham sites had bruchid damage. Much of the hemipteran damage occurred early in the development of the seeds. Location and cage were significant factors for both early (F = 20.24, df = 24, 780; P 0.0001; F = 283.23; df = 1, 780; P 0.0001) and late (F = 3.67; df = 24, 779; P 0.0001; F = 14.89; df = 1, 779; P 0.0001) hemiptera damage. Significant differences in damage caused by other insects were found by location (F = 3.62; df = 24, 779; P< 0.001) and the presence of a cage (F = 4.67, df = 1, 779; P < 0.05).

High levels of seed herbivory by one specialist (*B. ademptus*) and several generalists (primarily hemipterans) are widespread, and insects claim a high percentage of seeds. Tsugawa (1986) reported that less than 5% of kudzu seeds may be healthy, with variation in strains found within Japan. The best propagation method for kudzu appears to be through crown production with very little natural spread from seeds (Mes 1953, Tsugawa 1986). In the United States, spread is primarily through vegetative, not sexual reproduction (Sasek 1985). In a study of natural populations of kudzu in Japan, only vegetative reproduction was noted, with no reproduction from seed observed (Tsugawa and Kayama 1985). Abramavitz (1983) observed naturally occurring kudzu in Maryland and noted very little growth by first year plants, with only one of 245 surviving to the next year. With a high percentage of seeds lost to insect damage, and limited sexual reproduction, introduction of an additional seed herbivore would not be a useful strategy in a biological control program against kudzu.

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Literature Cited

Abramavitz, J.N. 1983. *Pueraria lobata* (Willd.) Ohwi, kudzu: limitations to sexual reproduction. M.S. thesis, University of Maryland, College Park, MD. 60 pp.

Bottimer, L.J. 1961. New United States records in Bruchidae, with notes on host plants and rearing procedures (Coleoptera). Ann. Entomol. Soc. Amer. 54: 291-298.

Dabadghao, P.M. 1949. Seed setting in kudzu vine *Pueraria thunbergiana*. Current Science. 18: 379-380.

Drought Monitor. National Drought Mitigation Service. Website: http://drought.unl.edu/dm/archive/99.

Duke, J.A. 1981. *Pueraria lobata* (Willd.) Ohwi. Pages 211-214 *in* Handbook of Legumes of World Economic Importance. Plenum Press. New York.

Mes, M.G.1953. Studies on the growth and reproduction of the kudzu vine (*Pueraria thunbergiana* (Sieb. and Zucc.) Benth.). S. Afr. J. Sci. 49:335-339.

Mitich, L.W. 2000. Kudzu [*Pueraria obata* (Willd.) Ohwi]. Weed Tech. 14: 231-235.

SAS Institute. 2001. SAS user's manual, version 8.2. SAS Institute, Cary, NC.

Tabor, P. 1942. Seed production by kudzu (*Pueraria thunbergiana*) in the southeastern United States during 1941. Amer. Soc. Agron.34: 389**.**

Tabor, P. and A.W. Susott. 1941. Zero to thirty million mile-a-minute seedlings. Soil Conservation. 7: 61-65.

Tsugawa, H. 1986. Cultivation and utilization of kudzu-vine. J. Japan. Grassl. Sci. 31: 435-443.

Tsugawa, H. and R. Kayama. 1985. Studies on population structure of kudzu vine (*Pueraria lobata* Ohwi). IV. The structure of overwintering aboveground parts of individual plants which constitute a natural kudzu population. J. Japan. Grassl. Sci. 31: 167-175.

Table 1. Kudzu seed collection sites across North Carolina, 1999.

County	Location	Date Caged
Alamance (1)	Near Snow Camp, Old Switchboard Rd nr jct Lindley Rd	20 August
Alamance (2)	Near Snow Camp, Griffin Rd nr Cane Creek	6 September
Alexander	NC Hwy 16, north of Taylorsville, east of road	18 August
Anson (1)	US 74, nr Pee Dee, south of rd, east of NC 145	24 August
Anson (2)	Near Lilesville, Old US 74, at SR 1740	24 August
Buncombe	S of Black Mtn, off NC Hwy 9	30 August
Catawba	US 70, SW of Catawba River bridge	18 August
Chatham	Near Siler City, off Raleigh St, W of town	20 August
Franklin	Near Louisburg, NC 561, N of jct NC 39	16 August
Granville (1)	Near Wilton, N of NC 56, W of jct NC 96	16 August
Granville (2)	Near Butner, Range Rd, NW of Butner Rd	1 September
Henderson	Near Broad River, NC 9 nr Old Fort Loop Rd	17 August
Hoke	Near McCain State Hospital, NC 211	24 August
Jackson	Near Balsam, nr jct US 23/74 & SR 1471	30 August
Johnston	NC 42, south of road between NC 39 & NC 222	20 August
Macon	Off US 23/74 behind Gold City Gem Mine	30 August
Nash	S of Spring Hope, NC 581, SE of Tar River	20 August
Orange	Near Jct of Crawford Dairy Rd & Old Greensboro Hwy	9 September

Table 1. (Cont.)

County	Location	Date Caged
Pitt (1)	E side Greenville, Hwy 43, S of Tar River	8 September
Pitt (2)	Near Bruce, NC 43 nr jct NC 121	8 September
Richmond	NC 74, W of Rockingham, next to Farm Bureau Office	24 August
Rockingham	Near Ruffin, NW of US 29 on Mayfield Rd	3 September
Wilkes	N of US 421, W of jct Sommers Rd (SR 2400)	18 August

Table 2. Comparison of insect damage to caged vs uncaged kudzu seed pods across North Carolina, 1999.

Location	Cage (-/+)	Total # pods	Total # seeds	Mean seeds/pod	% damaged	% bruchid	% Hemipteran early	late
Alamance	-	52	120	2.5 ± 1.7ijklmn	62.1 ± 45.9c	19.3 ± 37.3cde	39.0 ± 47.2efg	2.1 ± 14.4a
Alamance1	+	16	103	6.7 ± 3.1b	4.3 ± 10.8ab	0.8 ± 3.1a	2.3 ± 6.3a	1.2 ± 5.0a
Alamance2	+	15	43	2.9 ± 1.3hijk	0.0 ± 0.0a	0 .0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Alexander	-	24	132	2.4 ± 1.5 ijklmno	71.7 ± 46.7cde	30.7 ± 35.5ef	41.0 ± 36.7efgh	0.0a ± 0.0
Alexander	+	16	73	4.3 ±1.1defg	20.5 ± 28.0ab	18.9 ± 26.9bcde	1.6 ± 6.3a	0.0 ± 0.0a
Anson1	-	29	148	5.1 ± 2.0cd	92.7 ± 20.7ef	0.0 ± 0.0a	88.0 ± 27.2l	1.3 ± 4.7a
Anson1	+	27	246	9.1 ± 1.2a	1.7 ± 4.1ab	0.0 ± 0.0a	1.2 ± 3.4a	0.0 ± 0.0a
Anson2	-	20	93	4.6 ± 1.6def	80.3 ± 24.0cdef	7.7 ± 18.5abcd	48.5 ± 34.7fghi	21.1 ± 23.2c
Anson2	+	42	212	5.0 ± 1.8cde	3.8 ± 9.1ab	0.0 ± 0.0a	0.0 ± 0.0a	3.8 ± 9.1a
Buncombe	+	12	32	2.7 ± 0.8hijklm	2.8 ± 9.6ab	0.0 ± 0.0a	2.8 ± 9.6a	0.0 ± 0.0a
Catawba	-	18	16	1.6 ± 0.7klmnop	63.3 ± 48.3cd	0.0 ± 0.0a	63.3 ± 48.3hijk	0.0 ± 0.0a
Catawba	+	27	29	1.3 ± 0.4nop	15.2 ± 35.1ab	0.0 ± 0.0a	4.3 ± 20.9a	4.3 ± 20.9a
Chatham	-	8	18	2.3 ± 1.6ijklmno	97.5 ± 7.1f	26.9 ± 35.8ef	52.5 ± 43.7ghij	5.6 ± 10.5ab
Chatham	+	10	38	3.8 ± 1.8efgh	4.2 ± 9.0ab	4.2 ± 9.0abc	0.0 ± 0.0a	0.0 ± 0.0a
Franklin	+	37	68	1.8 ± 1.4jklmnop	21.9 ± 39.6b	0.7 ± 4.1a	10.8 ± 31.5abc	0.0 ± 0.0a

Table 2 (Cont.)

Location	Cage (-/+)	Total # pods	Total # seeds	Mean seeds/pod	% damaged[1]	% bruchid	% Hemipteran early	late
Granville1	-	12	37	3.4 ± 2.2fghi	83.3 ± 32.4cdef	6.1 ± 11.4abc	74.8 ± 35.0jkl	1.3 ± 4.3a
Granville1	+	47	126	2.7 ± 1.8ijklm	8.8 ± 23.5ab	1.4 ± 5.7a	6.3 ± 21.2ab	0.0 ± 0.0a
Granville2	-	8	25	3.1 ± 1.4ghij	89.6 ± 15.4ef	53.1 ± 4.9gh	36.5 ± 25.2defg	0.0 ± 0.0a
Granville2	+	17	47	2.7 ± 1.1hijkl	11.8 ± 19.3ab	4.9 ± 14.1abc	6.9 ± 15.4ab	0.0 ± 0.0a
Henderson	+	5	7	1.4 ± 0.5mnop	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Hoke	-	26	131	5.0 ± 1.8cde	98.6 ± 7.0f	2.7 ± 6.7ab	73.9 ± 27.1jkl	20.1 ± 26.5c
Hoke	+	8	26	3.3 ± 1.8ghi	20.2 ± 33.9ab	0.0 ± 0.0a	15.6 ± 35.2abcd	4.6 ± 8.5a
Jackson	+	6	6	1.2 ± 0.4op	80.0 ± 44.7cdef	0.0 ± 0.0a	80.0 ± 44.7kl	0.0 ± 0.0a
Johnston	-	29	180	6.1 ± 2.8bc	90.5 ± 16.6ef	56.0 ± 27.9gh	31.9 ± 32.4cdefg	2.6 ± 6.2a
Johnston	+	20	129	6.4 ± 1.8b	0.8 ± 3.7ab	0.8 ± 3.7a	0.0 ± 0.0a	0.0 ± 0.0a
Macon	+	5	7	1.4 ± 0.5mnop	20.0 ± 44.7ab	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Nash[2]	+	52	151	3.0 ± 1.6hij	88.3 ± 22.7ef	0.0 ± 0.0a	88.3 ± 22.7l	0.0 ± 0.0a
Orange	-	33	37	1.4 ± 0.6nop	96.8 ± 18.0f	24.7 ± 41.0ef	65.6 ± 43.9ijkl	6.5 ± 21.4bc
Orange	+	31	87	2.8 ± 1.4hijkl	21.5 ± 40.6b	0.0 ± 0.0a	19.9 ± 40.4abcde	0.0 ± 0.0a

Table 2 (Cont.)

Location	Cage (-/+)	Total # pods	Total # seeds	Mean seeds/pod	% damaged[1]	% bruchid	% Hemipteran early	% Hemipteran late
Pitt1	-	41	141	3.4 ± 1.1fghi	83.8 ± 27.1def	40.6 ± 32.6fg	40.7 ± 38.9efgh	0.0 ± 0.0a
Pitt1	+	18	56	3.1 ± 1.3ghij	6.9 ± 16.0ab	1.1 ± 4.7a	5.7 ± 14.2ab	0.0 ± 0.0a
Pitt2	-	7	11	1.6 ± 0.5lmnop	78.6 ± 39.3cdef	0.0 ± 0.0a	50.0 ± 40.8fghi	0.0 ± 0.0a
Pitt2	+	10	29	2.9 ± 2.1hij	12.5 ± 27.0ab	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Richmond	-	23	55	2.4 ± 1.3ijklmno	88.9 ± 23.1ef	22.8 ± 32.4de	50.5 ± 37.2fghi	13.0 ± 30.1c
Richmond	+	15	34	2.3 ± 0.9ijklmnop	18.9 ± 30.8ab	0.0 ± 0.0a	18.9 ± 30.8abcde	0.0 ± 0.0a
Rockingham	-	28	108	3.9 ± 1.9defgh	93.0 ± 15.2ef	58.5 ± 35.8h	27.7 ± 28.0bcdef	1.2 ± 6.3a
Rockingham	+	25	82	3.3 ± 1.8ghi	8.3 ± 22.8ab	5.0 ± 20.4abc	1.0 ± 5.0a	0.0 ± 0.0a
Wilkes	-	16	44	2.8 ± 1.2hijkl	84.4 ± 30.1def	32.3 ± 33.5ef	47.4 ± 31.4fghi	0.0 ± 0.0a
Wilkes	+	3	4	1.3 ± 0.6nop	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a

[1]Damaged by bruchids, hemipterans, or other insects.

[2]Cages were flooded during Hurricane Floyd, and damage from Tar River silt may have appeared as hemipteran damage.

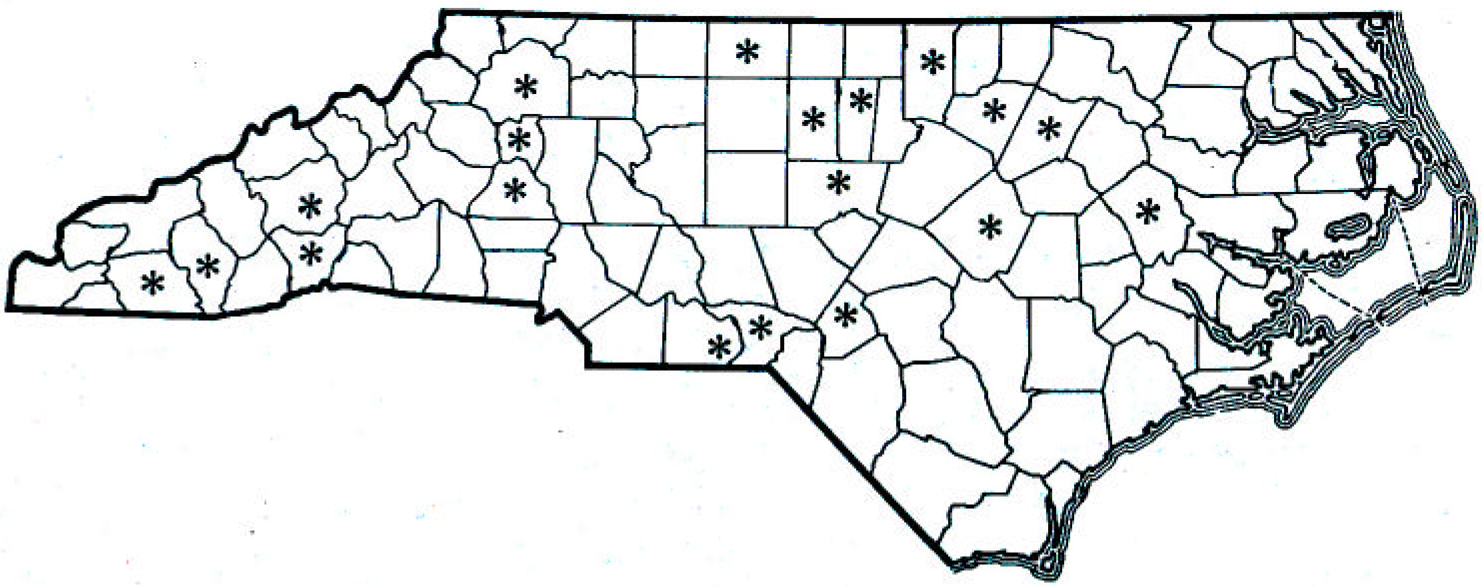

Figure 1. Kudzu seed collection sites across North Carolina, 1999. Counties where seeds were collected are marked with an *.

Chapter 3

Insect Herbivory on Kudzu Plants Grown from Seed Originating in China and the United States

This chapter has been submitted for publication in the Annals of the Entomological Society of America as: Kidd, K.A. and D.B. Orr. Insect Herbivory on Kudzu Plants Grown from Seed Originating in China and the United States.

Abstract

When plants are moved outside their native range and freed from their natural enemies, they may become weeds. *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.), kudzu, is widely distributed across the southeastern United States, but little is known about how plants in the USA compare to those in Asia, the native home of kudzu. Studies were conducted in the field and laboratory to compare feeding by native insects on plants grown from seeds collected in China and the USA. Throughout most of the field season, plants from China showed less insect defoliation than plants from the US. In a laboratory bioassay, however, no evidence was found that plants from China were better defended from herbivory than plants from the US. No significant differences were found in preimaginal development times of *Pseudoplusia includens* (Walker), our model herbivore, or in the amount of foliage consumed. By comparison, *P. includens* fed soybean foliage developed in significantly less time and were larger than those fed on kudzu from either source.

Keywords: kudzu, soybean, preimaginal development, EICA hypothesis, biological control

Kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & Almeida) is a common weed in the southeastern United States. Native to Asia, this plant is widely used in its native range for food and fiber (Duke, 1981). Kudzu was first introduced to the United States in 1876, at the Centennial Exposition in Philadelphia, and promoted as an ornamental plant and livestock feed (Mitich 2000). During the 1930s and 1940s kudzu was promoted for erosion control, and from 1935 to 1940, the US Soil Conservation Service made available 73 million kudzu seedlings to landholders for this purpose (Tabor and Susott 1941). Kudzu is now widely acknowledged as a major weed of forests and rights of way in the Southeast, and many herbicides are labeled for kudzu control on such sites (Miller and Edwards 1983). Because infestations may be extensive, remote, on marginal lands, or in environmentally sensitive areas where herbicide applications are not feasible, biological control of kudzu is being explored. To develop environmentally sound management plans for any weed, including biological control methods, an understanding of its basic biology including ecotypic or biotypic variation is necessary (Bhowmik 1997). Despite kudzu's pervasiveness, little is known of its ecology and interactions with native arthropods in the USA. Therefore, the objective of this study was to compare foliage feeding and development of arthropods on kudzu plants grown from seed of Chinese and US origin. This information will aid in determining if kudzu populations in the United States differ from populations from China, the center of kudzu's range in Asia, and may prove useful in the selection of natural enemies for future biological control programs.

Methods and Materials

During the fall of 1999, seeds were collected from > 20 locations in Anhui Province, China and pooled. Seeds also were collected from four sites in each of the three geographical regions of North Carolina, USA (coastal plain, piedmont and mountains). After damaged seeds were removed from samples, the remaining seeds were scarified by abrasion with coarse (80 grit) sandpaper and planted 0.6 cm deep in 12 cm diameter clay pots on 28 April 2000 (Kidd et al. 2002). Plants were grown in a greenhouse on the North Carolina State University campus. After plants had developed at least 10 true leaves and vines growth started, seedlings were planted in two separate fields at Umstead Research Farm near Butner, NC between 15 and 22 June to compare defoliation by insects in the field. Twelve plants from China and 12 from NC were planted in each block with 12.2 m spacing between plants. In each field, planting was done in a randomized complete block design with five blocks. Beginning 18 July, plants were examined biweekly to assess insect defoliation under field conditions. To estimate insect damage in the field, plants were compared to a chart of leaflets with known amounts of defoliation (0-100%, in increments of 10%), as measured with an area meter (Model LI-3100 Li-Cor, Lincoln, NE).

The nutritional quality of kudzu and its impact on the development and feeding by a model herbivore were examined in a laboratory bioassay. Additional kudzu plants from China and the US grown in the greenhouse as described above were transplanted into field plots adjacent to a soybean (var. Brim) plot (0.06 ha) on the NC State University Campus, and these plants (kudzu from two sources and soybean) were used in the bioassay. *Pseudoplusia includens* (Walker) was our model herbivore and individual larvae were reared separately on kudzu or soybean leaflets in feeding arenas as described by Kidd and Orr (2001). Neonate larvae from a laboratory colony were randomly assigned to feeding arenas (inverted, 150 by 15-mm plastic petri dishes) with a single leaflet of the test plant, and leaflets were changed every other day. Dishes were arranged in a completely randomized design with 30 replications, and were held at 25°C, 80% RH, and a photoperiod of 14:10 (L:D). Insects were examined daily, and each molt to a new stadium recorded. The number of days and number of stadia required to complete larval development were recorded. Insects were weighed to the nearest 0.1 mg with an analytical balance (Model M220D, Denver Instrument Co., Arvada, CO) within 24 h of pupation. When larvae reached the fifth stadium, foliage consumption estimates were made by measuring individual leaflets with an area meter before and after feeding. To compensate for different leaflet thickness, feeding rate was estimated as the consumption of dry matter. Mean dry matter for each plant species was determined at three points during the feeding trial, and the amount of leaf area consumed was converted to mg dry matter consumed using the mean dry matter value.

All data were analyzed using the general linear model procedure (PROC GLM, SAS Institute 1996). A two-way ANOVA with factors “host plant” and “sex” was conducted, followed by pairwise comparisons on least significant means for host plants. To achieve homogeneity of variance, pupal weights were log transformed.

Results and Discussion

In the field during most of the season, Chinese plants showed less insect defoliation than US plants (Table 1). However, on the last sampling date, no significant differences in defoliation were observed. Generalist herbivores, including grasshoppers (Orthoptera) and blister beetles (Coleoptera: Meloidae), were the predominant insect defoliators.

The laboratory bioassay with *P. includens* indicated that kudzu from China has similar nutritional qualities to kudzu from North Carolina for this insect (Table 2). Host plant (F = 85.46, df = 2, 84, P 0.001) and sex (F = 5.04, df = 1, 84, P 0.05) had significant effects on development time. The larval period was longer for insects feeding on kudzu, regardless of origin, compared to those feeding on soybean, and males feeding on Chinese kudzu took significantly longer to develop than females feeding on USA plants. The number of stadia required to complete development on kudzu, regardless of origin, was similar, and significantly fewer stadia were required for larvae to complete development on soybean foliage (F = 60.93, df = 2,84, P 0.001). Sex did not significantly affect the number of stadia (F = 1.68, df = 1, 84, P 0.05). Both host plant (F = 34.16, df = 2, 84, P 0.001) and sex (F = 7.90, df = 1, 84, P 0.001) significantly affected pupal weight. Insects feeding on kudzu were smaller than those feeding on soybeans, and female *P. includens* feeding on Chinese kudzu were significantly smaller than all others (F = 17.78, df = 5, 84, P 0.001). Sex and host plant were significant factors in the amount of dry matter consumed (F = 6.75, df = 1, 84, P 0.001and F=5.69, df = 2, 84, P 0.001, respectively). Males consumed more dry matter than females, and females feeding on soybean consumed the least (F = 4.14, df = 5, 84, P 0.001). These results are similar to those found in a previous study comparing feeding and development of *P. includens* on kudzu and soybean (Kidd and Orr, 2001).

The lower damage ratings in the field may be attributed to one or more factors. Ratings were based on the percentage of leaf area removed by herbivores, not the actual leaf area. Plants from China grow faster and are larger than those from the US (Kidd 2002), so new growth may offset the defoliation. Even if the same leaf area were removed per leaf, it would represent a smaller proportion of the total area. A laboratory analysis showed that the roots of Chinese plants contained more fiber than the US plants (Kidd 2002). If the foliage also contained more fiber, the plants may have been less palatable to foliar feeders (Scriber and Slansky 1981). This nonpreference may not have been evident in a no-choice feeding test, although the smaller size of the pupae, especially the females may support this idea.

The evolution of increased competitive ability (EICA) hypothesis proposed by Blossey and Nötzold (1995) predicts that non-native plants growing in the US should grow more vigorously and be less defended than those growing in their area of origin. If this hypothesis held true for kudzu, plants from the USA should be larger, but more susceptible to herbivory. Results of a growth study showed that plants originating in China grew approximately twice as large as plants from NC as measured by vine length, dry weight, and area covered by the plants (Kidd 2002). Some evidence of the Chinese plants being better defended, or at least less damaged, than the US plants is demonstrated in our field trial. Although data were highly variable on each sampling date, differences were highly significant. By the end of the season, defoliation was similar for plants from both sources. Before definitive conclusions may be drawn, a more precise quantification of defoliation in the field is needed. Further investigations into differences in foliar fiber and nutrient availability are merited to better understand the growth habit and ecology of kudzu from Asia and the USA.

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Literature Cited

Bhowmik, P.C. 1997. Weed biology: importance to weed management. Weed Sci. 45: 349-356.

Blossey, B. and R. Nötzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J. Ecol. 83:887-889.

Duke, J.A. 1981. Handbook of Legumes of World Economic Importance. Plenum Press. New York.

Kidd, K.A. and D.B. Orr. 2001. Comparative feeding and development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) on kudzu and soybean foliage. Ann. Entomol. Soc. Am. 94: 219-225.

Kidd, K.A. 2002. Comparative study of the growth of seedling kudzu originating in China and the United States. Ch 4, Ph.D. dissertation, North Carolina State Univerisity.

Miller, J. H. and B. Edwards. 1983. Kudzu: Where did it come from? And how can we stop it? So. J. Appl. For. 7: 165-169.

Mitich, L.W. 2000. Kudzu [*Pueraria lobata* (Willd.) Ohwi]. Weed Tech. 14: 231-235.

SAS Institute. 1996. SAS user's manual, version 6. SAS Institute, Cary, NC.

Scriber, J.M. and F. Slansky, Jr. 1981. The nutritional ecology of immature insects. Ann. Rev. Entomol. 26: 183-211.

Tabor, P. and A.W. Susott. 1941. Zero to thirty million mile-a-minute seedlings. Soil Conservation. 7: 61-65.

Table 1. Mean (± SD) insect defoliation (%) of Chinese and United States kudzu seedlings grown under field conditions. Butner, NC, 2000.

	Plant Origin		
Julian Date	China	USA	ANOVA
200	20.7 ± 21.9a	28.8 ± 23.9b	F = 7.31; df = 1, 214; P = 0.0001
214	22.6 ± 21.3a	36.4 ± 23.9b	F = 23.95; df = 1, 208; P = 0.0001
228	12.5 ± 17.0a	26.6 ± 24.6b	F = 22.44; df = 1, 190; P = 0.0001
244	17.5 ± 16.5a	26.3 ±18.9b	F = 12.09; df = 1, 190; P = 0.0006
257	12.6 ± 14.5a	18.1 ± 17.1b	F = 5.52; df = 1, 162; P = 0.0200
271	18.6 ± 22.4a	24.3 ± 24.0a	F = 2.79; df = 1, 162; P = 0.0969

Means within a row followed by the same letter are not significantly different (P>0.05, LSD).

Table 2. Mean (± SD) development time and foliage consumption by *Pseudoplusia includens* in the laboratory.

Host plant	Sex	n	Days to pupation	No. of stadia	Pupal weight (mg)[a]	Dry matter consumed (mg)
Kudzu						
China		20	18.8 ± 1.4bc	6.6 ± 0.6b	163.2 ± 40.8c	328.8 ± 94.7bc
		10	19.7 ± 2.0c	6.9 ± 0.6b	190.5 ± 20.2b	346.3 ± 30.9ab
USA		12	18.4 ± 1.2b	6.7 ± 0.5b	190.8 ± 30.5b	320.9 ± 53.2bc
		18	19.6 ± 2.6bc	6.7 ± 0.7b	207.6 ± 33.6b	388.4 ± 77.2a
Soybean		15	14.5 ± 0.9a	5.3 ± 0.5a	241.7 ± 32.8a	284.6 ± 51.2c
		15	14.5 ± 0.7a	5.3 ± 0.5a	256.6 ± 31.1a	316.2 ± 57.7c

Means within a column followed by the same letter are not significantly different ($P>0.05$), pairwise comparisons of least significant means.

[a]Data were transformed with the log transformation for analysis. Actual means are shown.

Chapter 4

Comparative Study of the Growth of Seedling Kudzu Originating in China and the United States

This chapter will be reformatted and submitted for publication to Weed Science as: Kidd, K.A., Orr, D.B, and J.C. Neal. 2002. Comparative study of the growth of seedling kudzu originating from China and the United States.

Abstract

Kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & Almeida) infests large areas of the southeastern USA, yet little is known about how these plants compare to kudzu growing in China, the center of its native range. As part of a larger project aimed at biological control of kudzu, comparisons of various first-year growth parameters, nutritional value, and susceptibility to insect herbivory were made of plants grown from seed collected in various locations in Anhui Province, China, and North Carolina, USA. Seedlings were grown in replicated tests at two sites in North Carolina. Kudzu from China was significantly larger than plants from the USA with average vine lengths of 149.85 cm and 87.71 cm, respectively. The allocation of resources for vine growth, as measured by the ratio of vine dry weight to area covered by the plant, was similar, but the ratio of root:above-ground biomass dry weight for plants of Chinese origin was 0.33 compared to 0.64 for North Carolina accessions. These data suggest seeds from China may have come from a different variety of kudzu than those from North Carolina.

Kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & Almeida) is a common weed in the southeastern United States. Native to Asia, the vine is widely used in China and Japan for food and fiber (Duke 1981, Mitich 2000, Shurtleff and Aoyagi 1977). It was first introduced to the United States in 1876, at the Centennial Exposition in Philadelphia and promoted as an ornamental and livestock feed (Mitich 2000). During the 1930s and 1940s kudzu was promoted for erosion control, and between 1935 and 1940, the US Soil Conservation Service distributed 73 million kudzu seedlings to landholders for erosion control (Tabor and Susott 1941).

Quantitative data are lacking, but anecdotal estimates suggest over 7 million acres in the southeastern US are infested (Everest et al. 1991), causing economic damage due to cost of control and lost forest product production. Kudzu is widely acknowledged as a major weed of forests and rights of way in the Southeast, and many herbicides are labeled for kudzu control in such sites (Kay and Monks 2002). Because infestations may be extensive, remote, or in environmentally sensitive areas where herbicide applications may not be feasible, biological control of kudzu is being explored. An important component of any weed control program is the study of the target weed's biology and ecology, including genetic diversity (Bhowmik 1997). Before any arthropod biological control agents are considered for introduction to the United States from Asia, it is important to develop a basic understanding of the ecology of kudzu and its associated arthropods in both countries. With such information the overall success of classical biological control programs against weeds may be improved (McClay 1995). Despite kudzu's pervasiveness, little is known of its ecology and interactions with native arthropods in the USA. The purpose of this study, therefore, was to determine if kudzu populations in the United States differ from populations in China, the center of kudzu's range in Asia. The specific objectives of this study were to compare the growth rate, biomass production and nutritional quality of kudzu plants from China and the United States.

Methods and Materials

Greenhouse Study

Seeds were collected from over 20 locations in Anhui Province, China (29°41' to 34°38' north, 114°51' to 119°37' east) and pooled. Seeds were also collected from four sites in each of three regions (coastal plain, piedmont, and mountains) of North Carolina, USA (33°50' to 36°36', north, 75°27' to 84°20' east). On 28 April 2000, after sorting for insect damage, seeds were scarified using coarse (80-grit) sandpaper and planted 0.6 mm deep in a soilless potting substrate (Metro Mix 200, The Scotts Co., Marysville, OH) in 12 cm diameter clay pots, and grown in a greenhouse at North Carolina State University. Mean temperature in the greenhouse was 25.1°C (range 17.5 to 36.6°C). Ambient light was supplemented on cloudy days with metal halide lights (1000 watt, 208 volt, Spero Electric Corporation, Cleveland, OH) for a minimum 13 h photophase. Controlled-release fertilizer (5.2 g of Osmocote 14-14-14, The Scotts Co, Marysville, OH) was added to each pot prior to planting. Pots were watered three times daily with an overhead mist irrigation system throughout the test.

To control error due to variability in light, temperature or humidity, pots were arranged on two benches in the greenhouse in a randomized complete block design with 12 plants from China and 12 (4 each from 3 regions) from NC in each of 17 blocks. The number of days to seedling emergence and the development of each true leaf were recorded until the plant had 10 leaves or was taken to the field.

Field Study

Seedlings from the greenhouse study were planted in two separate fields at Umstead research farm near Butner, North Carolina between 15-22 June 2000. Soil in both locations was Creedmoor coarse sandy loam (clayey, mixed, thermic Aquic Hapludults). Paraquat was applied 7 April at the rate of 0.5 kg ai ha^{-1}. Plots were marked and glyphosate was applied to 1 m^2 in the center of each plot on 5 June. Twelve plants from China and 12 from NC (4 sites from each of 3 regions) were planted 12.2 m apart in a randomized complete block design with five blocks in each field. Vine length and the number of leaves on each vine were recorded at planting.

To determine vine growth during the season, a single stem of each of the 24 plants in one plot per field was measured weekly beginning 14 July. Each stem selected originated at the crown of the plant, and was not branched. The stem was marked with flagging tape, and its length recorded weekly. If branching occurred during the growing season, all branches were measured and summed.

To estimate size and monitor growth during the season, all plants were measured biweekly beginning 18 July, using a 1 X 2 m grid, divided into 1 dm^2 squares. The grid was placed over individual plants, and each square containing any part of the plant was counted. The grid was moved as needed to cover all stems and leaves of the plant, and the person counting was positioned at the center of one of the 2 m sides of the grid. Counts were converted to m^2 and used to estimate the area covered by a plant. The spatial extent of the plant was recorded as the minimum area covered by the sampling grid when sampling each plant. To calculate the proportion of the spatial extent occupied by the plant (occupancy) the following equation was used.

$$\% \text{ Occupancy} = (C_A / E) \times 100 \quad [1]$$

C_A is the area covered by the plant as estimated with the sampling grid, and E is the spatial extent of the sampling grid.

Above-ground plant parts were harvested 6 and 9 October, bagged individually and returned to the laboratory and stored at 7.2°C. The number of vines growing from the crown of the plant was counted, and each vine was measured to its longest point. The number of secondary and tertiary branches and nodes was recorded. Internode length of each vine was calculated as the total length divided by the number of nodes. Vines were dried in a tobacco barn at 37.8°C for 10 days then weighed to the nearest 0.1 g. The cover:weight ratio (CWR) for each plant was calculated as:

$$CWR = C_{AF} / W_A \ (m^2\ g^{-1}) \quad [2]$$

C_{AF} is the area measurement (cover) obtained on the final sampling day, and W_A is the dry weight of the above-ground plant parts.

At harvest, a subsample of roots from the four accessions was taken from each block in both fields. Roots were washed and weighed to the nearest 0.1 g. Some roots were too small to analyze individually and therefore were pooled by origin within blocks for analysis. Roots were submitted to the Farm Feed Forage Testing Laboratory of the NC Department of Agriculture and Consumer Services for analysis. Protein was assayed following AOAC procedures (1990), and minerals and fat were analyzed following procedures developed at the forage lab (C. Fulk, personal comm.). Fiber was assayed with an Ankom 200 (Ankom Technology, 140 Turk Hill Park, Fairport, NY) according to the manufacturer's specifications. Carbohydrate content was calculated using the following formula (Van Soest et al. 1991).

$$\text{Carbohydrate (\%)} = 100 \quad CP \quad ADF \quad F \quad A \qquad [3]$$

All values were based on percent of dry matter. CP is crude protein, ADF is acid detergent fiber, F is fat, and A is ash (the sum of the minerals - calcium, phosphorus, sodium, magnesium sulfur, and potassium).

The ratio of root (R) to aboveground biomass (A) was calculated for those plants for which roots were sampled using the equation:

$$\text{R:A Ratio} = W_R / W_A \text{ (g g}^{-1}\text{)} \qquad [4]$$

W_R is the dry weight of the roots sampled, and W_A is the dry weight of the aboveground portion of the plant.

Statistical Analysis

Data for the greenhouse and field trials were subjected to a two-way ANOVA, followed by pairwise comparisons on least significant means for origin (PROC GLM, SAS Institute 2001). Trends were similar in both fields and there were few field by origin interactions, therefore data for the two field studies were pooled for analysis. To achieve homogeneity of variance in the field studies, the weights of roots and the total change in length for individual vines were log transformed. The season-long change in area covered and extent were square root transformed. Change in vine lengths and estimated area covered were fit to regression curves using the regression procedure and compared using a two-way analysis of variance (SAS Institute 2001).

Results and Discussion

Greenhouse Study

Seeds from China required longer to germinate than all North Carolina accessions ($F = 8.95$; df = 3, 313; P   0.001), although this may have been due to poor scarification (Table 1). The hard seed coat necessitates good scarification for germination (Tsugawa 1986). Plants from the coastal plain and mountain regions of NC emerged earliest, although piedmont seeds were not significantly different from mountain seeds. Kudzu seedlings from the coastal plain and mountains continued to develop ahead of China seedlings, but those from the piedmont were similar to those from China. Following emergence, growth rates for all accessions were similar, requiring about 13 days to reach the three-leaf growth stage and 30 days to six leaves, the point when the vine begins to form ($F = 1.25$; df = 3, 311; $P > 0.05$; $F = 1.43$; df = 3, 311; $P > 0.05$) (Table 1).

Field Study

Accurate rates of weekly vine growth over the season could not be calculated due to herbivory by deer and insects. If feeding occurred on the stem and not just the leaves, the vine branched. As secondary and tertiary vines formed, variances became larger, and measurements became less accurate (Table 2). Additionally, between 8 and 15 September, 10 plants in one field died due to excessive rain and poor drainage. However, some general trends can be seen when weekly measurements are examined through 8 September (Table 2) (Figure 1). Significant differences in the slopes of the regression lines for vine lengths were found (F = 85.93; df = 4, 24; P 0.001) (Table 3). Plants from China grew more rapidly than all other accessions with a slope almost twice that of accessions from the mountains or the piedmont of NC. Kudzu from the mountains and piedmont grew at rates similar to each other, while the growth rate for accessions from the coastal plain was approximately 28% of others from NC. However, total change in length from 7 July to 8 September was not statistically significant (F = 2.91; df = 3, 31; P > 0.05), probably due to variability within accessions.

In the field, kudzu vines from Chinese seeds grew significantly larger than kudzu grown from seeds collected in North Carolina when measured by several parameters (Tables 4-7, Figure 1-3). The grid sampling method was biased to overestimate the area covered, but provided a method to monitor the season-long growth habit of a sprawling vine. Spatial extent provides information on how far the plants spread out from the crown and occupancy describes the density of the vines and foliage in that space. Together, the three parameters provide a more accurate description of the growth habit of the four accessions.   When the area covered by the vines is examined, at the beginning of the measurements (18 July) only piedmont accessions are smaller than Chinese lines (F= 3.29; df = 3,190; P 0.05) (Table 4) (Figure 2).   By 30 August (44 days after first measurement), the piedmont lines were not different from the Chinese lines (F= 4.63; df = 3, 166; P > 0.05). North Carolina accessions were never significantly different from one another in area. Although mountain and coastal plain accessions were not initially different from Chinese lines, their slower growth rate became apparent after two to four weeks (Figure 2). Over the season the rate of change in the area covered, indicated by the slope of the regression line, was greater for Chinese accessions than all NC lines (F = 140.3; df = 4, 16; P 0.001) (Table 5) (Figure 2). Significant differences in the change in area covered (F = 4.73; df = 3, 107; P < 0.05) as well as the change in spatial extent over the measuring period were found (F = 4.60; df = 3, 107; P < 0.05) (Table 5). The difference in the rate of change in extent over the season was also significant (F = 91.3; df = 4, 16; P 0.001) (Table 5). No statistically significant differences in extent were found between plants at the biweekly measurements until 12 September (Table 6) (Figure 3). Significant differences in occupancy were found only at the first measurement (F = 3.59; df = 3, 190; P 0.001) and 28 days later (F = 2.91; df = 3, 166; P 0.001) (Figure 3). At the first measurement, occupancy by piedmont accessions was significantly smaller than Chinese accessions, and the other two NC lines were intermediate. Twenty-eight days later occupancy by mountain and piedmont accessions was significantly lower than that of Chinese accessions and coastal plain accessions were intermediate.

No differences were found in the number of vines at the crown (mean = 3.3) (F = 0.59; df = 3, 132; P > 0.05) (data not shown). Chinese plants had significantly longer total vine, more nodes, and greater internode lengths on each vine (F = 7.28; df = 3, 132; P 0.001; F = 8.59; df = 3, 132; P 0.001; F = 6.81, df = 3, 132; P 0.001) (Table 7). Chinese kudzu had significantly heavier above-ground biomass than plants from the NC coastal plain and mountains (F = 8.07; df = 3, 132; P 0.001). Interestingly, the cover:weight ratio (CWR) was not significantly different (F = 0.31; df = 3, 132; P > 0.05), an indication that the plants allocated their resources to the above-ground portions of the plant in the same proportions.

When individual roots were examined, no significant differences in weight were found, probably due to the high variability within accessions (F = 1.52; df = 3,69; P> 0.05) (Table 8). Differences were found, however in the ratio of root to shoot (above-ground biomass) dry weights (F = 13.11; df = 3, 67; P 0.001). Chinese plants had the lowest root:shoot ratio, indicating more of the available resources were directed to vine growth than to root production. Piedmont and coastal plain lines were not significantly different from each other.

Our analysis of the roots showed Chinese plants contained significantly more acid detergent fiber (ADF) and fewer digestible carbohydrates than US plants (F = 6.66, df = 3, 30; P 0.001; F = 6.42, df = 3, 25; P 0.001) (Table 8). Carbohydrate content is more accurately determined using neutral detergent fiber values, but for purposes of comparison in our study we used the more readily available ADF value. Crude protein levels were similar among accessions except those from the piedmont, which had slightly higher crude protein content than the Chinese accessions (F = 3.85, df = 3, 30; P 0.05). Corley et al (1997) reported the roots of kudzu to be 8.6% crude protein and 53.3% acid detergent fiber, but did not indicate the age of plants tested. it is reasonable to assume that the roots of older plants may be more fibrous than first year plants used in this study. Using a cold water extraction technique that yielded fiber content ranging from 10 to 19.5 g 100 g^{-1} dry weight, Achremowicz et al. (1993) demonstrated an increase in fiber content with the age of the root. In our study, the differences in fiber and starch content support the hypothesis that the plants may be two different varieties of kudzu, characterized in China as “water kudzu” or “fiber kudzu” with water kudzu used as a source of starch for food and/or traditional medicine.

Kudzu plants do not flower until they are about 3 years old (Tabor 1942, Dabadghao 1949). If water kudzu in China is preferentially harvested, only the fiber kudzu may be left long enough to produce seeds in the province where our collection was made. This activity may also have selected for high rates of vegetative growth. In the United States between 1935 and 1940, the Soil Conservation Service obtained enough seed from Japan to produce 73 millions seedlings, likely the source of most of the plants across the southeastern United States. Kudzu has been used in Japan for centuries for food and fiber and with time selection for different characteristics may have occurred.

The EICA hypothesis (Evolution of Increased Competitive Ability) proposed by Blossey and Nötzold (1995) and Blossey and Kamil (1996) uses the example of purple loosestrife (*Lythrum salicaria* L.) to suggest that invasive plants may evolve an increased competitive ability (vegetative growth) in new areas where they become established. Several studies have been conducted to confirm the EICA hypothesis, but none has conclusively demonstrated genetic changes when plants moved outside their native range (Willis and Blossey 1999, Willis et al. 1999, Willis et al. 2000). In a comparison of *L. salicaria* from native and non-indigenous populations grown in two common gardens (in Europe and the USA), Willis and Blossey (1999) found considerable inter-population variation, regardless of garden location. They suggested that rather than evolving post-invasion, the more vigorous genotypes displaced less-competitive conspecifics in the new location. To address the hypothesis that non-indigenous plants have fewer chemical defenses than indigenous plants, Willis et al. (1999) examined *L. salicaria* collected from various indigenous and non-indigenous populations. They found no significant effect of the origin of the plant on herbivore performance and no difference between populations in total leaf phenolic content.

To test the EICA hypothesis for additional species, Willis et al. (2000) examined four plant species native to Europe that had become established as weeds on other continents. After growing the plants in a common garden experiment, they found no evidence that non-indigenous plants were larger or directed more resources to reproduction than those from the native range. Thébaud and Simberloff (2001) used the literature to compare plants from Europe and the USA (California and the Carolinas). They looked at plant species introduced from Europe to California (n = 337) or the Carolinas (n = 189) and the reciprocal, species native to California (n = 40) or the Carolinas (n = 57) introduced to Europe. Plants native to Europe were not significantly taller in California or the Carolinas, but plants from the USA that had been introduced to Europe were significantly taller in their native range than in Europe, leading them to believe there was a “Europe effect.”

Our present study does not support the EICA hypothesis, but suggests that kudzu in North Carolina does not have increased vegetative growth. In fact, by the end of the growing season, plants grown from seeds collected in China were approximately 50% larger than those grown from locally collected seeds. On all sampling dates until 13 September, Chinese accessions showed significantly less feeding damage by generalist herbivores (primarily grasshoppers) than NC accessions (Kidd 2002). Yet, in the lab, no differences in development times or size were found for a generalist herbivore (*Pseudoplusia includens*) when fed kudzu from both sources, suggesting similar nutritional value (Kidd 2002).

One possible explanation for changes in plant vigor or defense in a new environment is artificial selection. Many of our worst weeds were originally introduced as ornamentals, nectar plants, or for other uses (Foy et al. 1983, Gordon and Thomas 1997 Reichard and White 2001). It is likely that these introductions were selected for a variety of characteristics including vigor, flowering ability or growth habit, and this selection may have enhanced their ability to invade natural areas. Kudzu seedlings from Soil Conservation Service nurseries were selected for planting based on root size, using the criteria "seedlings with one or more roots as large as an ordinary lead pencil" (Tabor and Susott 1941). This may have favored those plants that put more energy into root production than shoot production early in development. Between 1935 and 1940, the Soil Conservation Service obtained enough seed from Japan to produce 73 millions seedlings for distribution in the southeast (Tabor and Susott 1941). Kudzu has been used in Japan for centuries for food and fiber and with time selection for different characteristics may have occurred. As seed became difficult to obtain from Japan, crown production became the preferred method for propagation. A rapidly-growing, strongly-rooting variety, Kudzu-23, was identified in 1941, and propagated vegetatively for distribution by the Soil Conservation Service (Davis and Young 1951). The variety was described as having fine-textured foliage with small stems and leaflets, and hay that is more acceptable to livestock than coarser varieties. The fine-texture and greater acceptance by herbivores may be due to less fiber in the foliage. The original source of the kudzu growing in North Carolina is not known, but kudzu was first reported in NC in 1934 (Patterson 1976). By 1976 kudzu was present in 62 of 100 counties, so some undoubtedly came from the Japanese accessions. It is unknown how much, if any, originated from the

Kudzu-23 nursery. Further investigation into these questions is merited, especially examining known varieties of kudzu (water vs. fiber) in China, as well as other regions of its native range. Variation between and within North Carolina kudzu accessions, especially those from the piedmont, may also warrant further study to better understand the biology and growth habits of this plant in its introduced range.

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Literature Cited

Achremowicz, B, R.D. Tanner, A. Prokop, and R.D. Grisso. 1994. The effect of frost on the starch yield from kudzu (*Pueraria lobata*) roots of various ages grown in Northern Alabama. Bioresource Technology. 47: 149-151.

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis. 15th ed., 1st supplement. AOAC, Washington, DC.

Bhowmik, P.C. 1997. Weed biology: importance to weed management. Weed Sci. 45: 349-356.

Blossey, B. and R. Nötzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J. Ecol. 83:887-889.

Blossey, B. and J. Kamil. 1996. What determines the increased competitive ability of non-indigenous plants. Pages 3-9 *in* V.C. Moran and J.H. Hoffmann, eds. Proceedings of the IX International Symposium on Biological Control of Weeds, Stellenbosch, South Africa, 1996. University of Cape Town, Cape Town.

Corley, R.N., Woldeghebriel, and M.R. Murphy. 1997. Evaluation of the nutritive value of kudzu (*Pueraria lobata*) as a feed for ruminants. Anim. Feed Sci. Technol. 68: 183-188.

Dabadghao, P.M. 1949. Seed setting in kudzu vine *Pueraria thunbergiana*. Current Science. 18: 379-380.

Davis, R.L. and W.C. Young. 1951. Kudzu-23 – a new fine-textured variety. Soil Conservation. 14: 279-280.

Duke, J.A. 1981. *Pueraria lobata* (Willd.) Ohwi. Pages 211-214 *in* Handbook of Legumes of World Economic Importance. Plenum Press. New York.

Everest, J.W., J.H. Miller, D.M. Ball, M.G. Patterson. 1991. Kudzu in Alabama. History, uses, and control. Circ. ANR-65.8 pp.

Foy, C.L., Forney, D.R. and W.E. Cooley. 1983. History of weed introductions. Pages 65-92 *in* Wilson, C.L. and C.L. Graham, eds. Exotic Plant Pests and North American Agriculture. New York, NY: Academic Press.

Gordon, D.R. and K.P. Thomas. 1997. Florida's invasion by nonindigenous plants: history, screening, and regulation. Pages 21-37 *in* D. Simberloff, D.C. Schmitz, and T.C. Brown, eds. Strangers in Paradise. Impact and Management of Nonindigenous Species in Florida. Washington, D.C.: Island Press.

Kay, S.H. and D.W. Monks. 2002. Chemical control of specific weeds. Pages 406-408 *in* D.F. Ritchie, ed. 2002 North Carolina Agricultural Chemicals Manual. Raleigh, NC: North Carolina State University.

Kidd, K.A. 2002. Insect herbivory on kudzu plants originating in China and the United States. Ch 3, Ph.D. dissertation, North Carolina State University.

McClay, A. S. 1995. Beyond "before-and-after:" Experimental design and evaluation in classical weed biological control. Pages 213-219 *in* E.S. Delfosse and R.R. Scott [eds.], Proceedings, VIII International Symposium Biological Control of Weeds, Canterbury, NZ, 1992. CSIRO Publishing, Melbourne, AU.

Mitich, L.W. 2000. Kudzu [*Pueraria lobata* (Willd.) Ohwi]. Weed Tech. 14: 231-235.

Patterson, D.T. 1976. The history and distribution of five exotic weeds in North Carolina. Castanea: 41: 177-180.

Reichard, S.H. and P. White. 2001. Horticulture as a pathway of invasive plant introductions in the United States. BioScience. 51: 103-113.

SAS Institute. 2001. SAS user's manual, version 8.2. SAS Institute, Cary, NC.

Shurtleff, W. and A. Aoyagi. 1977. The book of kudzu. A culinary and healing guide. Autumn Press. Brookline, MA.

Tabor, P. 1942. Seed production by kudzu (*Pueraria thunbergiana*) in the southeastern United States during 1941. Amer. Soc. Agron.34: 389.

Tabor, P. and A.W. Susott. 1941. Zero to thirty million mile-a-minute seedlings. Soil Conservation. 7: 61-65.

Thébaud, C. and D. Simberloff. 2001. Are plants really larger in their introduced ranges? Am. Nat. 157: 231-236.

Tsugawa, H. 1986. Cultivation and utilization of kudzu-vine. J. Japan. Grassl. Sci. 31: 435-443.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Willis, A.J. and B. Blossey. 1999. Benign environments do not explain the increased vigour of non-indigenous plants: a cross-continental transplant experiment. Bio. Contr. Sci. and Tech. 9: 567-577.

Willis, A.J., J. Memmott, and R.I. Forrester. 2000. Is there evidence for the post invasion evolution of increased size among invasive plant species? Ecol.Lett. 3: 275-283.

Willis, A.J., M.B. Thomas, and J.H. Lawton. 1999. Is the increased vigour of invasive weeds explained by a trade-off between growth and herbivore resistance? Oecologia. 120: 632-640.

Table 1. Mean (± SD) emergence and leaf development of kudzu in a greenhouse.

Seed Origin	n	Days from Planting to: Emergence	Leaf 6	Days from Emergence to: Leaf 3	Leaf 6
China	171	14.3 ± 11.1c	43.4 ± 10.4c	12.5 ± 2.5a	28.8 ± 4.2a
North Carolina					
Coastal Plain	62	7.7 ± 4.8a	37.8 ± 8.3ab	12.6 ± 2.5a	30.1 ± 6.1a
Piedmont	52	10.9 ± 10.5b	41.0 ± 11.9bc	13.2 ± 3.0a	30.0 ± 7.3a
Mountains	53	8.7 ± 3.8ab	37.6 ± 6.7a	12.8 ± 2.2a	28.9 ± 4.7a

Means within a column followed by the same letter are not significantly different (P>0.05, LSD).

Table 2. Mean (± SE) vine length (cm) of kudzu seedlings grown under field conditions, Butner, NC, 2000.

	Mean length (cm)				
Date	China	NC: Coastal Plain	Piedmont	Mountains	ANOVA
14 July	62.4 ± 9.8a	23.8 ± 8.6b	25.6 ± 7.8b	40.3 ± 10.8b	F = 9.01; df = 3, 17; P = 0.0008
21 July	102.8 ± 14.1a	57.8 ± 14.6a	57.4 ± 14.1a	92.9 ± 24.3a	F = 0.83; df = 3, 17; P = 0.4944
28 July	146.8 ± 19.8a	84.6 ± 25.6a	106.5 ± 20.8a	111.6 ± 29.3a	F = 1.16; df = 3, 15; P = 0.3559
4 August	172.1 ± 34.85a	77.1 ± 22.5a	132.3 ± 33.2a	139.2 ± 38.5a	F = 0.96; df = 3, 14; P = 0.4397
11 August	227.3 ± 38.9a	57.1 ± 16.4a	124.8 ± 22.3a	184.6 ± 55.7a	F = 1.21; df = 3, 7; P = 0.3751
18 August	290.7 ± 61.6a	86.7 ± 20.8a	186.8 ± 66.0a	178.4 ± 68.8a	F = 0.93; df = 3, 8; P = 0.4686
25 August	305.9 ± 54.5a	80.4 ± 16.1a	223.0 ± 133.5a	200.0 ± 83.6a	F = 1.08; df = 3, 9; P = 0.4042
1 September	382.8 ± 90.2a	80.7 ± 3.2a	187.9 ± 96.0a	218.6 ± 98.6a	F = 1.47; df = 3, 10; P = 0.2818
8 September	366.2 ± 99.6a	105.0 ± 26.8a	207.6 ± 102.4a	239.2 ± 96.9a	F = 1.08; df = 3, 9; P = 0.4046

Means within a row followed by the same letter are not significantly different (P>0.05, LSD).

Table 3. Mean (± SD) change in length of individual stems of kudzu and regression of weekly measurements, 14 July to 8 September.

Source	n	Length Change (cm)[1]	SE	Regression[2]
China	14	421.1 ± 395.2a	105.6	Y = 62.4 + 5.9x, R^2 = 0.98a
North Carolina				
Coastal Plain	7	80.2 ± 57.1a	21.6	Y = 46.3 + 0.9x, R^2 = 0.59c
Piedmont	6	213.9 ± 264.6a	108.0	Y = 45.3 + 3.4x, R^2 = 0.89b
Mountains	5	201.6 ± 202.6a	90.6	Y = 63.5 + 3.3x, R^2 = 0.95b

Means within a column followed by the same letter are not significantly different (P>0.05, LSD).

[1]Data were transformed using the log transformation. Actual means are shown.

[2]Mean separation applies to slope of regression line.

Table 4. Mean (± SE) estimated area covered (m^2) by kudzu seedlings grown under field conditions, Butner, NC, 2000.

Date	Area covered (m^2)				ANOVA
	China	NC: Coastal Plain	Piedmont	Mountains	
18 July	0.22 ± 0.01a	0.20 ± 0.02ab	0.17 ± 0.02b	0.18 ± 0.02ab	F = 3.29; df = 3, 190; P = 0.0218
1 August	0.41 ±0.03a	0.36 ± 0.02ab	0.34 ± 0.04ab	0.30 ± 0.03b	F = 2.95; df = 3, 184; P = 0.0339
15 August	0.61 ± 0.05a	0.38 ± 0.05b	0.43 ± 0.07b	0.38 ± 0.05b	F = 4.60; df = 3, 166; P = 0.0040
30 August	0.84 ± 0.07a	0.48 ± 0.07b	0.61 ± 0.11ab	0.50 ± 0.08b	F = 4.63; df = 3, 166; P = 0.0039
12 September	0.93 ± 0.10a	0.47 ± 0.09b	0.72 ± 0.14ab	0.43 ± 0.09b	F = 7.14; df = 3, 138; P = 0.0020
27 September	1.14 ± 0.14a	0.48 ± 0.12b	0.82 ± 0.17ab	0.48 ± 0.11b	F = 6.92; df = 3, 138; P = 0.0002

Means within a row followed by the same letter are not significantly different (P>0.05, LSD).

Table 5. Change in the estimated area covered and spatial extent of kudzu plants 18 July to 27 September, 2000, Butner, NC. Mean (± SD) and regression of biweekly measurements.

Origin	n	Area (m^2)[1]	SE	Regression[2]	Extent (m^2)[3]	SE	Regression[2]
China	79	1.0 ± 1.2a	0.1	Y = 23.3 + 1.3x, R^2 = 0.99a	6.2 ± 9.2a	1.0	Y = 1.7 + 0.08x, R^2 = 0.98a
North Carolina							
Coastal Plain	18	0.5 ± 0.6b	0.2	Y = 24.5 + 0.4x, R^2 = 0.87c	2.3 ± 5.9b	1.4	Y = 2.1 + 0.02x, R^2 = 0.94c
Piedmont	27	0.7 ± 0.9ab	0.2	Y = 18.7 + 0.9x, R^2 = 0.99b	4.1 ± 6.7ab	1.3	Y = 1.8 + 0.05x, R^2 = 0.94b
Mountains	19	0.5 ± 0.6b	0.1	Y = 23.5 + 0.4x, R^2 = 0.80c	1.7 ± 4.1b	0.9	Y = 2.1 + 0.01x, R^2 = 0.78c

Means within a column followed by the same letter are not significantly different (P>0.05, LSD).

[1]Data were transformed using the square root transformation. Actual means are shown.

[2]Mean separation applies to slope of regression line.

[3]Data were transformed using the log transformation. Actual means are shown.

Table 6. Mean (± SE) estimated spatial extent (m^2) of kudzu seedlings grown under field conditions, Butner, NC, 2000.

	Spatial Extent (m^2)				
Date	China	NC: Coastal Plain	Piedmont	Mountains	ANOVA
18 July	2.1 ± 0.0a	2.0 ± 0.1a	2.0 ± 0.0a	2.0 ± 0.0a	F = 0.50; df = 3, 190; P = 0.6800
1 August	2.7 ± 0.1a	2.6 ± 0.3a	2.4 ± 0.5a	2.2 ± 0.1a	F = 0.91; df = 3, 184; P = 0.4371
15 August	3.7 ± 0.3a	2.5 ± 0.3a	2.9 ± 0.3a	2.7 ± 0.2a	F = 2.56; df = 3, 166; P = 0.0566
30 August	4.7 ± 0.4a	3.1 ± 0.7a	4.0 ± 0.7a	2.9 ± 0.3a	F = 2.45; df = 3, 166; P = 0.0654
12 September	5.9 ± 0.7a	3.5 ± 0.8b	4.1 ± 0.7ab	2.6 ± 0.3b	F = 5.28; df = 3, 138; P = 0.0018
27 September	7.7 ± 1.0a	3.6 ± 1.0b	5.7 ± 1.2ab	3.1 ± 0.6b	F = 5.15; df = 3, 138; P = 0.0021

Means within a row followed by the same letter are not significantly different (P>0.05, LSD).

Table 7. Final vine measurements of kudzu grown near Butner, NC, 2000 (Mean ± SD).

Origin	n	Vine Length (cm)	# Nodes	Internode Length (cm)	Est. Cover (m^2)	Total Wt (g)[1]	Cover: Wt Ratio
China	85	149.9 ± 86.6a	12.9 ± 4.7a	10.7 ± 3.5a	1.1 ± 1.3a	53.9 ± 70.3a	2.7 ± 1.2a
North Carolina							
Coastal Plain	27	80.3 ± 57.3b	8.6 ± 4.3b	8.7 ± 2.9b	0.5 ± 0.7a	20.2 ± 18.6b	2.8 ± 1.2a
Piedmont	29	96.6 ± 74.6b	9.6 ± 3.8b	8.9 ± 4.2b	0.8 ± 0.9a	35.3 ± 47.2ab	2.5 ± 0.7a
Mountains	28	85.6 ± 52.6b	9.9 ± 3.7b	8.4 ± 4.1b	0.5 ± 0.6a	19.9 ± 25.6b	2.7 ± 0.9a

Means within a column followed by the same letter are not significantly different (P>0.05, LSD).

[1]Data were transformed to log (weight). Actual means are shown.

Table 8. Root analysis of kudzu plants grown near Butner, NC, 2000 (Mean ± SD).

Origin	n	Harvested Wt (g)[1]	% Dry Matter	Root: Shoot Ratio	% Crude Protein	% ADF	% Carbohydrates
China	25	66.1 ± 65.2a	19.3 ± 2.3b	0.3 ± 0.2c	11.2 ± 1.9b	42.1 ± 5.9a	42.0 ± 7.0b
North Carolina							
Coastal Plain	11	59.3 ± 53.0a	21.7 ± 4.3a	0.7 ± 0.3a	12.4 ± 2.7ab	33.4 ± 10.9b	53.8 ± 6.7a
Piedmont	10	78.2 ± 85.7a	21.6 ± 2.2a	0.7 ± 0.4a	13.6 ± 2.1a	33.7 ± 5.1b	48.8 ± 5.7a
Mountains	9	30.4 ± 36.3b	21.7 ± 2.9a	0.5 ± 0.2b	12.5 ± 2.5ab	32.5 ± 9.5b	48.8 ± 6.8a

Means within a column followed by the same letter are not significantly different ($P>0.05$, LSD).

[1]Data were transformed with the log transformation for analysis. Actual means are shown.

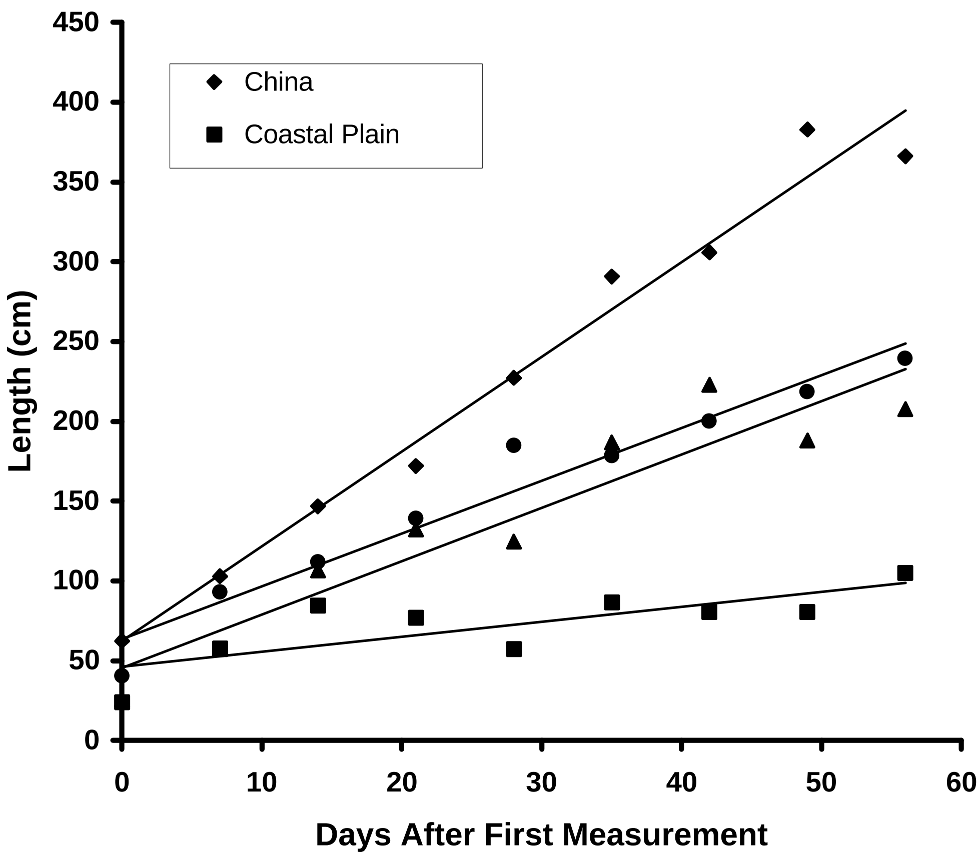

Figure 1. Mean lengths of individual stems of kudzu from Anhui Province, China and three regions of North Carolina, USA. Values represent means of pooled data from two fields. The regression equations for the rate of change within the season are: China = 62.4 + 5.9x, R^2 = 0.98; Piedmont = 45.3 + 3.4x, R^2 = 0.89; Mountains = 63.5 + 3.3x, R^2 = 0.95; Coastal Plain = 46.3 + 0.9x, R^2 = 0.59.

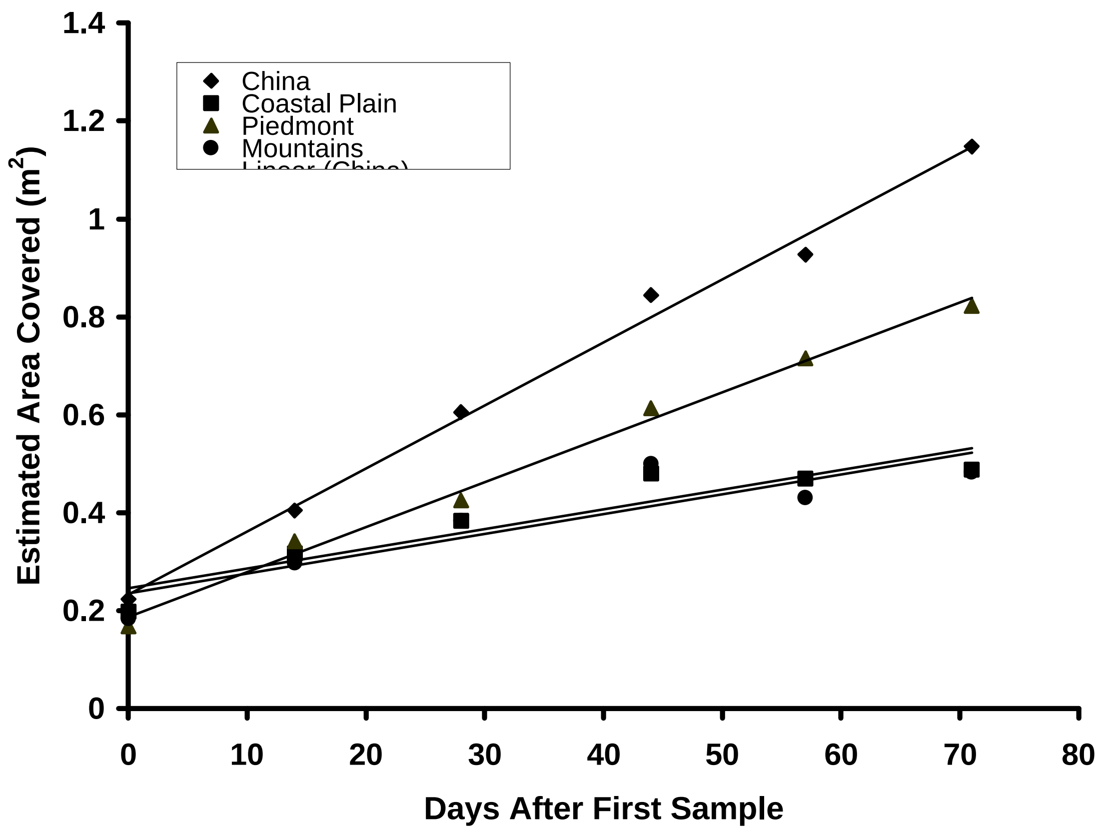

Figure 2. Estimated area (m^2) covered by kudzu. Rates of increase for each accession are represented by the equations: China = 23.3 + 1.3x, R^2 = 0.99; Piedmont = 18.7 + 0.9x, R^2 = 0.99; Mountains = 23.5 + 0.4x, R^2 = 0.80; Coastal Plain = 24.5 + 0.4x, R^2 = 0.87.

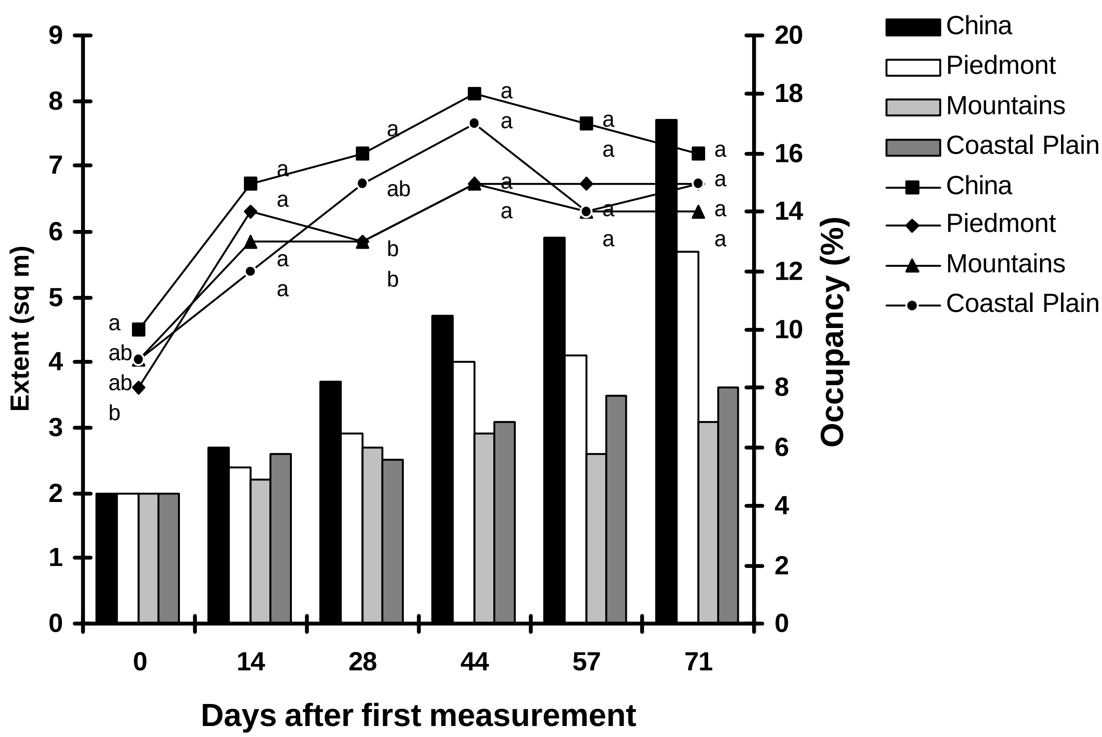

Figure 3. Extent (m^2) and occupancy (%) of kudzu plants. Values on a given date with the same letter are not significantly different (P>0.05, LSD).

Chapter 5

Volatile Production by Kudzu

in Response to Herbivory

and Its Effects on Predation

Abstract

Plants emit volatile chemicals that serve a variety of ecological functions including defense from herbivores. Volatile chemicals produced in response to herbivory may provide direct defense, or they may provide indirect defense, serving as semiochemicals to attract enemies of the herbivores. This indirect defense has been documented in a variety of field crop and natural systems.

Preliminary studies indicate that kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) produces volatiles in response to herbivory that increase the rate of predation by generalist predators, primarily *Polistes* spp. (Hymenoptera: Vespidae). Feeding by the general herbivore *Pseudoplusia includens* (Walker) resulted in an increase in visits by predators at certain times of the year. The same effect was not noted when damage was produced mechanically, nor was frass from the insects attractive to predators.

Solid phase microextraction (SPME) and dynamic headspace methods were used to collect the volatiles produced by kudzu in the laboratory. Samples were analyzed by gas chromatographic methods, but only preliminary results were obtained.

Plants produce and emit a variety of volatile chemicals, serving many ecological functions, including compounds that are released in response to herbivory (Paré and Tumlinson 1999, Farmer 2001, Hunter, 2002). These compounds may provide a direct defense (i.e. deterring feeding) or they may serve as semiochemicals, attracting the herbivore's predators and parasitoids, and plants under attack have been demonstrated to become more attractive to these natural enemies (Paré and Tumlinson 1999, Farmer 2001). The emission of herbivore-induced volatile chemicals by plants has been studied in numerous systems, including apple, corn, cotton, and bean (Takabayashi et al. 1991, Agelopoulos and Keller 1994, Turlings 1995, Paré and Tumlinson 1997, De Moraes 1998, Turlings et al. 1998). Specific volatiles are emitted as a response to herbivore attack and an elicitor for these chemicals has been isolated from oral secretions of herbivores (Paré and Tumlinson 1999, Kessler and Baldwin 2002). For in-depth reviews of this subject, see Chadwick and Goode (1999).

In the laboratory, dynamic headspace trapping methods have been used to collect headspace volatiles for analysis (Heath and Manukian 1992, 1994, Agelopoulos and Keller 1994, Turlings et al. 1998). With this system, air is forced through a chamber containing the plant material, then through a trap containing an adsorbent material such as Tenax or Porapak. Volatiles are then extracted using methylene chloride or other solvents and injected into a gas chromatograph (GC) or a gc-mass spectrometer (GC-MS) for analysis. Solid phase microextraction (SPME) is a solventless technique, using a coated fiber to adsorb volatiles (Pawliszyn 1997). Desorption is accomplished directly in the injector of the GC or GC-MS. A newer technique, it has primarily been used for food, flavor, and fragrance applications (Field et al. 1996, Clark and Bunch 1997, Jia et al. 1998, Hayasaka and Bartowsky 1999, An et al. 2001). When comparing the two techniques Elmore et al. (1997) found similar profiles for the two methods, although a smaller response for the SPME, and concluded that SPME could give satisfactory results for major components.

As part of a biological control program, the soybean looper, *Pseudoplusia includens* (Walker) has been released on kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & Almeida) (Kidd and Orr 2001). These releases have been observed to result in the attraction of a large number of generalist predators (D.B. Orr, personal comm.). Preliminary studies showed that neither mechanical damage nor the application of frass to kudzu foliage increased the activity of predators in test plots (K.A. Kidd, unpublished data). Based on these observations, we hypothesized that feeding by the caterpillars resulted in the emission of volatiles that were attractive to predators. Much of the previous work on volatile production has been conducted in the laboratory, although increasingly more is being conducted in the field (Hunter 2002). The objectives of this study were to determine if volatile chemicals that attract predators are produced by kudzu in the field as a response to herbivory, and to isolate those chemicals in the laboratory.

Methods and Materials

Field study

To determine if herbivory on kudzu increases the activity of predators, especially *Polistes* sp. wasps, five field plots were established on the Centennial Campus of North Carolina State University. Plots were 1 m^2 in area, separated by 4 m. The test was replicated 5 times using a Latin square design to control error due to learning by the predators and the movement of volatiles between plots. The treatments were: 1) 100 lab-reared soybean looper (*P. includens)* larvae in the 5th stadium; 2) artificial wounding (injury simulation); 3) artificial wounding with the application of regurgitant to the holes; 4) application of a frass extract from loopers which had fed on kudzu for 24 hr; and 5) untreated control. Treatments were applied one hour in advance of data collection to allow predators to locate the plots and establish searching activity. All larvae for treatment 1 were released in the plot and allowed to redistribute themselves on the foliage. For the second treatment, 50 holes (a quantity estimated to equate the feeding of 100 caterpillars for one hour) were punched in leaves throughout the plot with a single-hole paper punch. For treatment 3, 50 holes were punched and caterpillars were held with their mouthparts at the wound and gently squeezed to obtain regurgitant. Frass collected from caterpillars feeding on kudzu leaves in the laboratory was mixed with water to form a slurry, and was applied to foliage in treatment 4 once, distributing it on the foliage as evenly as possible. Predator activity was measured by direct observation, as counts of wasps and other predators coming to plots for two one-hour observation periods. After the first observation period, 50 additional holes were punched in treatments 2 and 3, and regurgitant applied in treatment 3. The second observation was made one hour later.

Field data was analyzed using the general linear models procedure of the Statistical Analysis System (SAS 2001). An ANOVA using the factors plot, treatment, and replication was carried out. To achieve homogeneity of variance, data for Observations 1 and 2 were log transformed.

Laboratory Study

To determine the origin and identity of volatiles, samples of headspace volatiles were collected from foliage that had been fed upon by loopers in the laboratory. For comparison, volatiles were collected from undamaged, control leaves and from artificially wounded leaves. Two methods of volatile collection were tested. A solid phase microextraction (SPME) sampler was used to collect volatiles in either a 4 liter glass jar with a septum in the lid or a Tedlar™ gas collection bag (61 cm X 61 cm) fitted with a PTFE/silicone septum valve. An excised stem supported in a glass, water-filled test tube was placed in the jar for the sampling period. The bag was used for whole potted kudzu seedlings. The bag was split across one side, and the aboveground portions of the seedling were enclosed inside it. The bag was held closed with two sliding plastic clips (28.5 cm long) and metal binder clips. After allowing the plant material one-hour equilibration time, the SPME sampler was inserted through the septum, and exposed for 1 - 30 hours to determine the optimum sample time. Volatiles were desorbed in the injection port of a HP-5890A gas chromatograph at 225ºC with a 30 m x 0.25 mm (ID) fused silica column with a 0.25 μm thick bonded methyl silica stationary phase. The initial oven temperature was 75ºC, heated to 250ºC at 5ºC/min. Final temperature was held for 20 minutes.

The dynamic headspace volatile collection chamber consisted of a large glass preparative column (1 m x 10 cm ID) with tubing connectors at both ends. A 6-port valve with flanged Teflon® tubing and 0.6 cm-28 threaded fitting was connected to the effluent tubing connector, and volatile collection tubes were attached to each of the 6 ports using Teflon tubing adapters (0.6 cm OD x 0.15 cm ID) and Tygon® tubing. The volatile collection tubes were adsorbent tubes (0.1 cm OD x 10 cm) packed with 80/100 mesh Porapak QS (Supelco, Bellefonte, PA) in two beds, 200 and 100 mg each, separated with silanized cotton wool plugs. The chamber was pressurized with highly purified air moving at approximately 2000 ml/min.

Adsorbent tubes were preconditioned by washing with HPLC-grade methylene chloride. Either an exised kudzu stem supported in a water-filled test tube or a kudzu seedling in a 10 cm square plastic pot were infested with fourth stadium larva of *P. includens* and placed in the volatile collection chamber, taking care not to damage the foliage. After plant material had been in the chamber for 24 hours the first port was opened to collect volatiles on the Porapak QS adsorbent. After volatiles were collected in adsorbent tubes, beds were separated and volatiles extracted with methylene chloride. Porapak from both beds was combined in a graduated centrifuge tube, and cotton wool placed in a separate tube. Methylene chloroide (10 ml) was added, and the material was vortexed 1 min at 15 min intervals for 1 hr. Tubes were capped and left overnight, and after 24 hours, the supernatant was decanted to a clean test tube and reduced under N to 1 ml. A 0.1 ìl sample was injected into the GC, using the temperature program above.

Results and Discussion

Field Study

Variable results were obtained in the field, but significant differences were found between treatments for Observation 1 (F = 5.15; df = 4, 12; P = 0.0119) and Observation 2 (F = 5.44; df = 4, 12; P = 0.0098) (Table 1). No differences were found for the change in number between observations (F = 2.21; df = 4, 12; P = 0.1296). Although the response in the mechanical + regurgitant plot was not as strong as the release plot, it was not significantly different. During the first one-hour observation period, only the frass and control plots were different from the release plot, but by the second observation period, the mechanically damaged plot was also visited less frequently than the release plot (Figure 1). Problems with the laboratory colony of *P. includens* prevented returning to the field until September and results were less dramatic.

Significant differences were found between replications (Obs. 1: F = 2.74; df = 4, 12; P = 0.0791, Obs. 2: F = 4.13, df = 4, 12; P = 0.0248). The last replication was not significantly different from the 1st and 2nd replications. Significantly fewer predators were counted during both observation periods of the 3rd and 4th replications than during the same periods of the 1st replication. During the first replication 28 June 2001 and the second replication 5 September, high numbers of predator visits were recorded in the insect release plot, with little activity in other plots. Yellowjackets (*Vespula* spp.) were the most common predator in September, not *Polistes.* On 7 September when the test was run two days after the previous replication, yellowjackets were frequently observed searching in the mechanical + regurgitant treatment plot that had most recently been the release plot. Although none were seen, some caterpillars may have remained in the plot, volatiles, if they were produced, may have still been present, or the predators may have been relying on learning more than on olfactory cues. Sabelis and Van De Baan (1983) reported that leaves remained attractive to predaceous mites for several hours after spider mites were removed. A type of associative learning occurs for anthocorids (Drukker et al. 2000). These predators responded to herbivore-induced volatiles after experience with them, but laboratory reared insects showed no response to the same odors. *Polistes* has a tendency to return to habitats where they have had success and colonies may develop hunting patterns (Rabb and Lawson 1957). During one observation period, we noted a wasp repeatedly land on a leaf where a looper had been caught and begin searching from that point. Although we can not be sure it was the same individual, it always came from the same direction and left in the same way. Because of these problems, the 5th replication was run on 6 June 2002. During the last replication, the released caterpillars did not appear to be feeding very much, and less predator activity occurred. In fact, a large *Polistes* was engaged in nest building just centimeters outside the plot, and did not leave her nest during the observation period.

Predation by *Polistes* may be seasonal, that is, early in the summer, they require protein for brood rearing, but late in the season, they forage for more carbohydrates to prepare for the winter. Stored honey has been found in *Polistes* nests in the fall (Gillaspy 1986). Additionally, during the September observations, kudzu was in flower, emitting a strong grape-like bouquet. This odor may have masked volatiles produced in response to herbivory; when the caterpillars feed on excised leaves in the lab, a similar, but fainter odor can be detected. From our results, we could not conclude that volatiles are emitted by kudzu and serve as semiochemicals. Further study is warranted, to determine if the increase in predation is due to cumulative volatile production, or learning by the wasps (or both). If the plant does emit these volatiles in response to lepidopteran feeding, it would seem that releasing a foliar feeder as a biological control agent would be futile. However, Turlings et al. (1998) showed that not all herbivores induce the production of volatiles. In their study, feeding by the aphid *Rhopalosiphum maidis* (Fitch) did not induce volatile production in corn. A leaf feeder, *Spodoptera littoralis* (Boisduval) and a stem borer *Ostrinia nubilalis*

(Hubner) both induced volatile production, although stem boring resulted in lower levels of chemicals. As part of any biological control program, the effect of predators or parasitoids should be determined in advance of any large-scale release of biocontrol organisms. If herbivore-induced volatiles that attract large numbers of generalist or host-specific predators could be identified, it may become possible to screen potential control agents early in the testing.

Laboratory Study

Due to numerous technical problems, only preliminary results were obtained. If the quantity of volatiles emitted from seedling kudzu is low, the dynamic headspace technique may be preferred over SPME. Given the response of predators in the field and the ability of a human to detect a change in odors after insect feeding on kudzu in the lab, further work is warranted to better understand kudzu-herbivore interactions.

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Literature Cited

Agelopoulos, N.G. and M.A. Keller 1994. Plant-natural enemy association in tritrophic system, *Cotesia rubecula-Pieris rapae*-Brassicaceae (Cruciferae). III. Collection and identification of plant and frass volatiles. J. of Chem. Ecol. 20: 1955-1967.

An, M., T. Haig, and P. Hatfield. 2001. On-site field sampling and analysis of fragrance from living lavender (*Lavendula angustifolia* L.) flowers by solid-phase microextraction coupled to gas chromatography and ion-trap mass spectrometry. J. Chromatogr. A 917: 245-250.

Chadwick, D.J. and J.A. Goode [eds]. 1999. Insect-Plant Interactions and Induced Plant Defence. Novartis Foundation Symposium. Wiley and Sons, New York, NY.

Clark, T.J. and J.E. Bunch. 1997. Qualitative and quantitative analysis of flavor additives on tobacco products using SPME-GC-Mass-Spectroscopy. J. Agric. Food Chem. 45: 844-849.

De Moraes, C.M., W.J. Lewis, P.W. Paré , H.T. Alborn, and J.H. Tumlinson 1998. Herbivore-infested plants selectively attract parasitoids. Nature 393: 570-573.

Drukker, B., J.Bruin, and M.W. Sabelis. 2000. Anthocorid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. Physiol. Entomol. 25: 260-265.

Elmore, J.S., M.A. Erbahadir, and D.S Mottram. 1997. Comparison of dynamic headspace concentration on Tenax with solid phase microextraction for the analysis of aroma volatiles. J. Agric. Food Chem. 45: 2638-2641.

Farmer, E.E. 2001. Surface-to-air signals. Nature. 411:854-856.

Field, J.A., G. Nickerson, D.D. James, and C. Heider. 1996. Determination of essential oils in hops by headspace solid-phase microextraction. J. Agric. Food Chem. 44: 1768-1772.

Gillaspy, J.E. 1986. *Polistes* wasps: Biology and impact on man. *In*: Vinson, S.B., [ed.]. Economic Impact and Control of Social Insects. pp 332-351. Praeger, New York, NY.

Hayasaka, Y. and E.J. Bartowsky. 1999. Analysis of diacetyl in wine using solid-phase microextraction combined with gas chromatography-mass spectrometry. J.Agric. Food Chem. 47: 612-617.

Heath, R.R. and A. Manukian. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plants using a charcoal-infused medium for air purification. J. Chem. Ecol. 18: 1209-1226.

Heath, R.R. and A. Manukian. 1992. An automated system fro use in collecting volatile chemicals released from plants. J. Chem Ecol. 20: 593-608.

Hunter, M.D. 2002. A breath of fresh air: beyond laboratory studies of plant volatile-natural enemy interactions. Agric. and For. Entomol. 4:81-86.

Jia, M., Q.H. Zhang, and D.B. Min. 1998. Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. J. Agric. Food Chem. 46: 2744-2747.

Kessler, A. and I.T. Baldwin. 2002. Plant responses to insect herbivory: the emerging molecular analysis. Anu. Rev. Plant Biol. 53: 299-328.

Kidd, K.A. and D.B. Orr. 2001. Comparative feeding and development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) on kudzu and soybean foliage. Ann. Entomol. Soc. Am. 94: 219-225.

Paré , P.W. and J.H. Tumlinson 1997. De Novo biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol. 114: 1161-1167.

Paré , P.W. and J.H. Tumlinson 1999. Plant volatiles as a defense against insect herbivores. Plant Physiol. 121: 325-331.

Pawliszyn, J. 1997. Solid Phase Microextraction: Theory and Practice. Wiley. New York, NY.

Rabb, R.L. and F.R. Lawson. 1957. Some factors influencing the predation of *Polistes* wasp on the tobacco hornworm. J. Econ. Entomol. 50: 778-784.

SAS Institute. 2001. SAS User's manual, version 8.2. SAS Institute, Cary, NC.

Takabayashi, M.D.J., M. Dicke, and M.A. Posthumus. 1991. Variation in composition of predator-attracting allelochemicals emitted by herbivore-infested plants: relative influence of plant and herbivore. Chemoecology 2: 1-6.

Turlings, T.C.J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz and S. Dorn. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: Possible consequences for their natural enemies. Biological Control 11: 122-129.

Turlings, T.C.J., J. H. Loughrin, P.J. McCall, U.S.R. Röse, W.J. Lewis. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc. Natl. Acad. Sci. USA 92: 4169-4174.

Table 1. Visits by predators during one-hour observation periods to kudzu plots, Raleigh, NC, 2001-2002. (Mean ± SD).

Treatment	n	Observation 1[1]	Observation 2[1]	Change
Release	5	6.4 ± 6.5a	17.0 ± 18.9a	10.6 ± 13.7a
Mechanical Damage	5	3.0 ± 2.3ab	2.2 ± 2.3bc	-0.8 ± 1.5a
Mechanical + Regurgitant	5	4.8 ± 3.8a	7.4 ± 7.0ab	2.6 ± 6.0a
Frass	5	1.0 ± 0.7bc	1.2 ± 1.3c	0.2 ± 1.3a
Control	5	0.8 ± 1.3c	1.2 ± 1.3c	0.4 ± 1.1a

Means within a column followed by the same letter are not significantly different ($P < 0.05$, LSD)

[1]Data were transformed with the log transformation ($\log(x + 1)$) prior to analysis. Actual means are shown.

Table 2. Number of predators visiting kudzu plots, Raleigh, NC, 2001-2002. Total counts for each one-hour period (Observation 1, Observation 2).

Date	*Polistes* spp	*Vespula* spp	Coccinellids	Spiders	Other
28 June 01	11, 47	0, 0	4, 3	8, 5	5, 3
5 Sept 01	0, 0	20, 38	0, 1	0, 2	2,1
7 Sept 01	0, 0	6, 18	0, 0	0, 3	0, 2
10 Sept 01	0, 0	0, 1	1, 0	2, 3	5, 0
6 June 02	5, 19	0, 0	5, 3	0, 0	3, 0

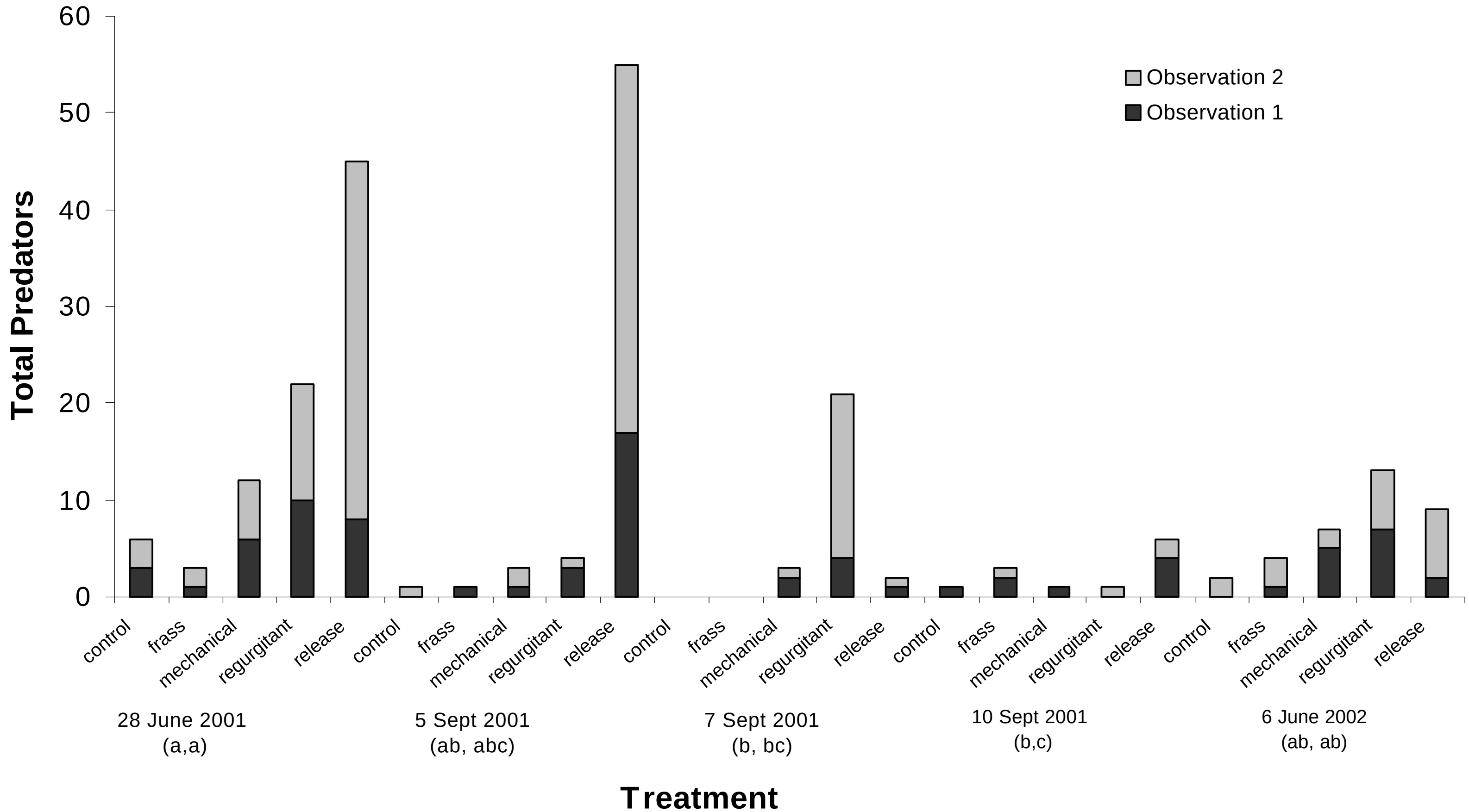

Figure 1. Total number of predators visiting kudzu plots, Centennial Campus, NCSU, 2001-2002. Mean separation (LSD, P< 0.05) for observations (1, 2) within replications.