BIOLOGY AND HOST SPECIFICITY OF *GONIOCTENA TREDECIMMACULATA* (COLEOPTERA: CHRYSOMELIDAE): A POTENTIAL BIOLOGICAL CONTROL AGENT FOR KUDZU

by

Matthew J. Frye

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Matthew J. Frye

Approved:_____
Judith A. Hough-Goldstein, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
Douglas W. Tallamy, Ph.D.
Chairperson of the Department of Entomology and Wildlife Ecology

Approved: _____
Robin W. Morgan, Ph.D.
Dean of the College of Agriculture and Natural Resources

Approved: _____
Conrado M. Gempesaw II, Ph.D.
Vice Provost for Academic and International Programs

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ABSTRACT

Gonioctena tredecimmaculata (Jacoby) (Coleoptera: Chrysomelidae) was sent from China to the United States for testing as a potential biological control agent of kudzu [*Pueraria montana* var. *lobata* (Willd.) Maesen & S. Almeida]. In a quarantine facility, females kept on kudzu produced 2-4 larvae per day by ovoviviparous reproduction during most of the summer. Insect development was rapid, with larval and pupal stages taking 5.6 ± 0.08 and 9.6 ± 0.13 days at 25 °C, respectively. Larvae consumed a total of 16.3 ± 0.63 cm^2, while adult beetles consumed approximately 5 cm^2 of kudzu foliage per day. Newly emerged adults fed on foliage for approximately 15 days before digging into the soil for an apparent obligate diapause. These beetles mated and reproduced the following spring.

The host range of this insect was tested using several native and agriculturally important plants related to kudzu under no-choice conditions. Both adults and larvae rejected most of the plants that were tested, but fed on soybean [*Glycine max* (L.) Merr.] and American hog-peanut [*Amphicarpaea bracteata* (L.) Fernald] in addition to kudzu. In a related study, host specificity tests investigated the response of *G. tredecimmaculata* to the growing condition of kudzu and soybean. Field- and greenhouse-grown foliage were used to determine if variable plant traits alter the response and specificity of a potential biological control agent in quarantine studies. Field foliage of both plants

exhibited greater leaf toughness, higher total carbon content, higher trichome density per mm^2, and reduced water content compared to greenhouse foliage. However, these traits did not influence insect fecundity, development time, female oviposition preference, or adult choice for field vs. greenhouse foliage of kudzu or soybean. Therefore, feeding and reproduction by *G. tredecimmaculata* on an agriculturally important plant, soybean, has led to its rejection as a candidate for biological control of kudzu. This work suggests that, despite significant differences in plant traits, greenhouse-grown foliage may be acceptable for use in host specificity testing.

Chapter 1 - Biology and host range of *Gonioctena tredecimmaculata*

Introduction:

Kudzu, *Pueraria montana* var. *lobata (*Willd*.)* Maesen & S. Almeida, was first introduced to the United States in 1876 at the Centennial Exposition in Philadelphia (Mitich 2000), and again in 1883 at the New Orleans Exposition (Everest et al. 1991). Although it currently grows more prolifically in the Southeastern United States than anywhere else in the world, this plant is native to Asia (Shurtleff & Aoyagi 1985). Taxonomically, kudzu belongs to the Phaseoleae, a tribe within the Fabaceae (Leguminosae) defined by a twining habit, trifoliolate leaves with asymmetrical lateral leaflet margins (Bruneau et al. 1994), stipules at the base of petioles, and stipels at the base of petiolules (Viviani et al. 1991). Specifically, kudzu belongs to the Glycininae, considered a polyphyletic subtribe due to similarities between its members and plants in other Phaseoleae subtribes (Viviani et al. 1991, Bruneau et al. 1994, Kajita et al. 2001).

Although kudzu has been used extensively in China and Japan for many centuries as a food crop, medicinal herb, basket making material, and for making paper (Shurtleff & Aoyagi 1985), it was originally advocated in the United States for its sweet-smelling blossoms and as a porch cover to create shade (Everest et al. 1991). As early as 1910, kudzu had gained recognition as a drought-resistant, high-nitrogen forage crop (Britton et al. 2002), and was used for livestock pasturage, fodder and hay in the South (Mitich

2000). During the 1930's and 1940's kudzu was heavily prescribed for its use as a cure for erosion, which thrust kudzu into the public eye (Mitich 2000). In the first five years following this designation, it had been introduced into every southern state, and the Soil Conservation Service had produced over 73 million seedlings for distribution (Tabor & Susott 1941). These early efforts to introduce and spread kudzu produced a high degree of genetic variation and clonal diversity in U.S. populations (Pappert et al. 2000, Jewett et al. 2003). Several varieties of kudzu were introduced, and the morphology of reproductive structures (van der Maesen 1985) as well as molecular techniques (Sun et al. 2005) can be used to distinguish among group members.

As early as 1955, the push for kudzu had come to a halt, as people were quickly disenchanted with the vine (Shurtleff & Aoyagi 1985). Two problems arose that earlier advocates had not foreseen. First, kudzu climbs over existing foliage and kills trees and saplings via light competition (Wechsler 1977, Munger 2002). Second, there is no practical way to stop it from taking over the landscape (Blackwell 1973). Thus, in 1970, kudzu was classified as a common weed, and by 2000 it was deemed a noxious weed in Florida, Kansas, Oregon, Pennsylvania, Washington, and West Virginia. In the time since then, it has been responsible for thousands of dollars worth of lumber and pulpwood damage, as well as costs to keep the vine off of rights of way and power lines (Shurtleff & Aoyagi 1985). A current and pressing issue regarding the widespread distribution of kudzu in the U.S. is the recent introduction of *Phakopsora pachyrhizi* Sydow, the causal agent of soybean rust. The potential of this disease to overwinter on kudzu poses a threat to agriculture, since infestation of soybean from kudzu foliage could

occur annually and cause severe losses of soybean crops (Perez-Hernandez et al. 1994, Pivonia & Yang 2004).

Despite the economic losses attributed to kudzu, some members of the scientific community continue to promote the usefulness of this plant. One position taken by such proponents is that kudzu's hardiness would make it a useful commercial crop, as the negative issues resulting from natural enemies, disease, fertilizer use, and irrigation are minimized (Parks et al. 2002). It has also been suggested that plant biomass be used for the production of renewable fuels such as ethanol, while starch from kudzu rhizomes could be harvested based on the benefits it has over other forms of starch (Tanner et al. 1979, Parks et al. 2002). The use of kudzu in the phytoremediation of heavy metals (Brown et al. 2001, Conell & Al-Hamdani 2001) and the absorption of basic dyes in wastewaters produced by the textile industry (Allen et al. 2005) have been investigated as well. The most recent use proposed for kudzu deals with the medicinal or herbal properties of the plant. Studies with rats (Benlhabib et al. 2004) and humans (Lucas et al. 2005) have illustrated the potential for kudzu extracts to reduce alcohol consumption, and future work is anticipated. However, the useful qualities that this plant may exhibit in certain situations do not make kudzu a beneficial organism. In fact, its rapid growth and capacity to spread designate kudzu as a potential threat to biodiversity (Forseth & Innis 2004). The following sections highlight past and present efforts to reduce the negative impact of this invasive vine.

In the fifty years since kudzu has been considered a pest, few methods have been identified to control or eradicate this alien weed. Furthermore, the current measures that

provide control are often expensive and slow to produce results. Overgrazing by cattle and persistent weeding or mowing have been proposed as possible techniques to eliminate kudzu, and preliminary results using Angora goats as grazers show some promise (Bonsi et al. 1991). The use of these techniques is based on the observation that complete defoliation over several growing seasons depletes kudzu root reserves, which is one of very few ways to kill the plant (Brender 1961). These treatments, however, are not practical for steep hillsides or locations where the vine has grown up and over trees (Blackwell 1973). Mechanical removal of roots is difficult and expensive, and chemical applications are generally ineffective unless they are combined with other control methods (Thomas 2000, Harrington et al. 2003). This includes the application of herbicides to the cut end of a stub or root-crown (Thomas 2000), or growing herbicide tolerant species capable of out-competing kudzu for light resources after controlled vegetation burning (Harrington et al. 2003). Nevertheless, these methods do not allow for large-scale control of kudzu, and the lack of mechanical and chemical control makes this invasive weed a target for classical biological control (Britton et al. 2002).

The idea that alien plants experience reduced regulation by herbivores in their introduced range, which results in increased growth and reproduction by the plant, is the foundation of classical biological control (Keane & Crawly 2002). By reuniting a plant with specialist herbivores from its native range, the plant is forced to allocate resources away from reproduction or growth and towards defense. As a result, the plant is less competitive and less capable of spreading at alarming rates (Blossey & Notzold 1995). With specific reference to kudzu, the argument for biological control through the use of

natural enemies is strengthened by the fact that 116 phytophagous insects in 6 feeding guilds were reported on kudzu in China (Sun et al. 2006), where it is not considered a pest species. In the U.S., surveys of kudzu infestations indicated a general lack of herbivores, comprising only 3 feeding guilds, and these insects were regarded as generalist feeders (Thorton 2004).

In 1999, a cooperative program funded by the United States Department of Agriculture Forest Service was initiated in China to survey natural enemies of kudzu and conduct initial host range tests. While field evaluations are beneficial in illustrating an insect's behavioral host range in its native habitat, the results of these tests often suggest a host range that is narrower than the insect's true physiological host range (McEvoy 1996). For this reason, potential biological control agents are shipped to quarantine facilities in the introduced range of the target weed to determine the specificity of an insect under no-choice feeding conditions. This practice establishes the maximal extent to which an agent can impact potential hosts (McEvoy 1996). The chrysomelid beetle, *Gonioctena tredecimmaculata* (Jacoby), was therefore shipped to the quarantine facility at the United States Department of Agriculture – Agriculture Research Service (USDA-ARS) Beneficial Insects Introduction Research laboratory (Newark, DE) to be evaluated as a biological control agent.

Limited research is available on the biology of *Gonioctena tredecimmaculata* from its native range, which includes China and Taiwan (Bezděk 2002). However, as a genus, *Gonioctena* Chevrolat (Coleoptera: Chrysomelidae: Chrysomelinae) is often cited for its (ovo)viviparity, defined as embryonic development that is completed, at least in

part, before larval deposition (Kudo et al. 1995). Larvae in several *Gonioctena* species are enclosed in a thin, opaque membrane from which they emerge shortly after deposition on a leaf surface (Takizawa 1976, Mason & Lawson 1982, Kudo & Ishibashi 1995, Kudo et al. 1995). Larvae feed on host foliage and pupation occurs in the soil (Takizawa 1976). Studies concerning several species of *Gonioctena* report these insects as univoltine, although diffuse adult emergence from hibernation over several months (Waloff & Richards 1958), asynchronous larviposition (Mason & Lawson 1982), and iteroparous reproduction (Kudo et al. 1995) can be confused with multivoltinism. In those species that have been studied, *Gonioctena* exhibit a short active life cycle in the first year of life, with newly emerged, sexually immature adult insects feeding for a brief time before entering the soil for hibernation (Waloff & Richards 1958, Takizawa 1976, Mardulyn et al. 1997). These adults will then emerge the following year in late spring and copulate after reaching sexual maturity (Waloff & Richards 1958).

Recent phylogentic analysis of *Gonioctena* has shown that the Fabaceae is the ancestral host of this genus (Mardulyn et al. 1997). However, eight shifts to distantly related and chemically dissimilar plant families have occurred over time (Mardulyn et al. 1997). It is suspected that these host shifts may have been directed towards plant species available in the insect's environment (Mardulyn et al. 1997), although most studies indicate that plant chemistry is the determining factor in host shifts (Beccera 1997). Despite these shifts, only one species [*G.* (*Spartomena*) *fornicata*] within the genus is recorded as a pest. This insect feeds on leguminous alfalfa crops (*Medicago sativa* L., Mardulyn et al. 1997). In any case, extreme caution should be taken in host specificity

testing before a new species is imported for biological control, as this would limit the possibility of a negative host shift. This can be accomplished by understanding completely the physiological host range of an insect. Therefore, the following study was undertaken to determine the ability of *G. tredecimmaculata* to feed and reproduce on several native or agriculturally important plants related to the target weed, and to understand this insect's biology. These are the first steps in validating the safety and potential efficacy of an insect being considered for introduction as a weed biological control agent.

Materials and Methods:

Test Plants

Individual kudzu plants from recently rooted nodes were collected from Dover (39° 10.943' N, 75° 32.764' W) and Glasgow (39° 37' 11.66" N, 75° 44' 42.20" W), Delaware in May and June of 2004, respectively. These plants were transported to the University of Delaware greenhouse in a large Tupperware bin and transplanted into plastic pots (30.5 cm diameter) with Pro-mix (Premier Horticulture, Red Hill, PA). Subsequent plants were grown in the greenhouse by the wave method, in which "a series of nodes are left above ground while one, two or three nodes are buried between them," producing a new plant at each buried node (Shurtleff & Aoyagi 1985). Foliage from a kudzu patch located in Fair Hill, Maryland (39° 42' 14.83" N, 75° 47' 59.39" W) was used later in the season. Trifoliate leaves were collected from this site with pruning sheers and transported to the laboratory in a self-sealing plastic bag.

Test plants chosen for host specificity trials were selected based on their taxonomy, as well as their agricultural or economic importance (Birdsall et al. 2004). Seeds of bush bean and pole bean (two varieties of *Phaseolus vulgaris* L.; Blue Lake 274 and Kentucky Wonder Brown, respectively) were purchased from K-Mart, sensitive joint-vetch [*Aeschynomene virginica* (L.) Britton et al.; AY104A] was purchased from Seedland.com (Wellsborn, FL), and seeds of both soybean [*Glycine max* (L.) Merr.; CL48RR] and hairy cowpea [*Vigna luteola* (Jacq.) Benth.] were obtained from Clark's Seed Inc. (Kent, DE). Plantings were made once a week to ensure that no-choice host tests would be conducted on plants of similar age, and plants were grown in 15 cm diameter plastic pots with Pro-mix. American hog-peanut [*Amphicarpaea bracteata* (L.) Fernald] was collected from the field at White Clay Creek State Park (Newark, DE), and trifoliate leaves were transported from the field to the laboratory in plastic self-sealing bags. Greenhouse plants were watered twice daily by drip irrigation, and grown under a light regime of 16L : 8D at approximately 27 ˚C. Plants were fertilized at recommended rates every 10 days with MiracleGro Liquid All Purpose Plant Food. Supplemental light was provided by a 1,000-W metal-halide lamp.

Insects

Biology & Rearing: The leaf feeding beetle, *G. tredecimmaculata*, was collected on kudzu in Xuancheng, Anhui Province, China. About 65 adult insects were packaged in insulated containers with kudzu foliage, and sent by Dr. Jiang-Hua Sun (Chinese Academy of Sciences; Beijing, China) to the USDA-ARS Beneficial Insects Introduction Research (BIIR) facility (Newark, DE) in June 2004. The 48 insects that survived the

shipping process were kept in the quarantine facility under a light regime of 16L : 8D at 25.6 ± 0.02 ˚C. Digital pictures of *G. tredecimmaculata* were sent to Jan Bezděk at the Mendel University of Agriculture and Forestry in the Czech Republic to confirm the identity of the insect.

The adult insects initially shipped to BIIR were kept in screen cages (47 X 41 X 35 cm) from June to October 2004, with one or two potted kudzu plants per cage. Kudzu plants were changed regularly to provide insects with ample foliage. However, due to a limited supply of potted kudzu and the insects' preference for young leaflets, kudzu foliage was later collected from the field every other day. Young trifoliate leaves were harvested and transported directly to the laboratory, where they were placed in aqua-picks to keep them fresh.

Efforts were made to identify differences between male and female *G. tredecimmaculata*, with a variety of external morphological characters examined. To test the predictive ability of certain characters, dissections were made to expose the reproductive anatomy of insects. Fecundity of adults was determined when new larvae were collected each day from adult cages (larviposition generally occurred overnight or early in the morning). Larvae were removed from kudzu foliage with a fine tip paintbrush and transferred to a plastic tub (32 X 26 X 10 cm; Pioneer Plastics Inc., Dixon, KY) for rearing. Each rearing tub contained ~ 7.5 cm Pro-mix as substrate, which was kept moist by spraying the soil with distilled water. Larvae were added to tubs for 8 consecutive days, with kudzu foliage added for a further 6 days (14 days total). This method was developed in order to maximize the number of larvae added to a tub before

adults began to emerge. Newly emerged adults were collected daily from rearing tubs, counted, and transferred to a separate screen cage containing only individuals reared in the laboratory. Survival to adulthood was determined as the number of adults that emerged divided by the number of larvae added to rearing tubs.

In early October 2004, a step-down process was initiated to overwinter the beetles, which was adapted from a protocol developed by Dan Palmer (Phillip Alampi Beneficial Insect Rearing Lab, New Jersey Department of Agriculture). Insects were counted, placed in self-sealing plastic bags containing moistened sphagnum moss, and moved from rearing conditions at 25 ˚C to a 13 ˚C chamber for 14 days. Following this period, insects were kept in a 1.7 ˚C chamber for approximately 220 days. In the spring of 2005, insects were taken from 1.7 ˚C and placed in 13 ˚C for 14 days. They were then placed in the 25 ˚C room on 16 June 2005 and each insect was removed from the sphagnum moss with soft forceps. Insect survival from overwintering was recorded. All of the tests described below were conducted during June, July and August 2004.

Adult Kudzu Consumption and Time to Diapause: Six pairs of newly emerged adult beetles were placed in plastic tubs (17 X 12 X 6 cm; Pioneer Plastics Inc., Dixon, KY) and provided with excess kudzu foliage for 14-17 days. This was done to quantify the amount of foliage consumed and the amount of time before adults burrow into the soil as part of an obligate diapause. To determine foliage consumption, leaves were collected from each of the 6 plastic tubs after insect feeding and individual leaflets were press-dried, photocopied and scanned into the computer as black and white images (HP Scanjet 3970; Hewlett-Packard Company, Palo Alto, California). ImageJ Software (ImageJ 1.36

for Windows; National Institutes of Health, Bethesda, MD) was used to determine leaf area in all cases, except when all the available leaf material was consumed. Excluding these instances, missing portions of pressed leaves were recreated with a black permanent marker, and the area of these images was determined as the total leaf area. The difference between the total leaf area and the leaf area remaining after insect feeding was recorded as insect foliar consumption. Feeding per pair per day and feeding per individual per day were estimated from total leaf consumption. To further investigate the amount of foliage consumed per day by single insects, seven newly emerged adult beetles were placed individually in 10 X 1.5 cm disposable plastic Petri dishes lined with filter paper. Distilled water was sprayed in dishes daily to keep filter paper moist and adults were given a single kudzu leaflet every day for seven days. Leaf area was analyzed by the method described above.

Adult No-Choice Tests: Newly emerged adult beetles, reared as larvae on kudzu, were used to test the physiological host range and oviposition of *G. tredecimmaculata*. Each replicate contained three pairs of adults on a host plant, which was enclosed in a 33 X 15 cm cylindrical cage of 0.12 cm gauge plastic film (Grafix Clear-Lay Plastic Film; Grafix Plastics, Cleveland, OH). Nylon netting was attached to the top of the cage to limit condensation on cage walls, and stapled seams were sealed with clear packing tape. Host plants or foliage were changed every 4 days. Potted plants were used for pole bean, bush bean, joint-vetch, cowpea and soybean, while fresh cut trifoliate leaves were used for kudzu and hog-peanut. Control replicates were pots containing Pro-mix and a dental wick saturated with water. Percent survival to diapause, percent survival over the winter

(to 2005), the time until death for non-survivors, and the amount of foliage eaten were recorded where possible. Survival and consumption are reported for individual insects, since no differences were found among replicates.

Larval No-Choice Tests: Naïve larvae were collected from kudzu foliage in adult rearing cages and placed individually in 10 X 1.5 cm Petri dishes lined with moistened filter paper (naïve insects are defined as larvae with no prior foliage consumption before being used in an experiment). Larvae were provided with excess leaflets of pole bean, bush bean, cowpea, kudzu, soybean, or hog-peanut, while a dish with moistened filter paper only served as a control. New leaflets were added every other day, while old leaflets were removed and press-dried for determining larval leaf area consumption using the method described above. Dishes were monitored daily for larval molts, as evidenced by the presence of head capsules. Once fourth instars began wandering, they were transported to 163 ml soufflé cups (Solo Cup Company, Highland Park, IL) with Pro-mix for pupation. Pupation cups were sprayed with distilled water daily to maintain soil moisture and protect pupating insects from desiccation. Time as pupae was recorded starting from the point when larvae were added to the soufflé cups and ending with adult emergence.

Larval Choice Tests: Naïve larvae were placed individually in Petri dishes lined with moist filter paper and presented with similarly sized leaflets of both kudzu and soybean. Larvae were placed on the filter paper and had equal access to a leaflet of either host plant, which overlapped in the middle of the dish. Larval development was determined

by daily monitoring of head capsules, and time as pupae was recorded. Leaf area consumption was measured for each host plant. Results are reported for insects that survived to pupation.

Data Analysis

The SAS System (Version 9.1; SAS Institute Inc., Cary, NC) was used for all statistical procedures. Data from the larval no-choice host specificity test were analyzed using ANOVA followed by Tukey's Studentized Range (HSD) Test, and a Paired t Test was used to compare foliage consumption from the larval choice test.

Results:

Insects

Biology and Rearing: Beetles reared under quarantine conditions exhibited a high reproductive rate, with 5 adult females producing 272 larvae in a 28-day period (approximately 2 larvae per insect per day). Females were noted as (ovo)viviparous, with eggs laid on leaf surfaces hatching shortly after deposition. Eggs were 2 - 3 mm in length and appeared as a thin membrane surrounding the developed larvae. When observed, eggs were found on the adaxial (upper) leaf surface with the anterior end of the insect facing up.

Larval and adult feeding of *G. tredecimmaculata* were distinct, with larvae producing holes in the center of a leaf and adults feeding from the edge of a leaf inward. Larvae passed through four instars in 5.6 ± 0.08 days (n = 30) and the pupal stage lasted 9.6 ± 0.13 days (n = 30). Larvae reared in plastic tubs exhibited a 71% adult emergence rate (273 of 383) and 85% of the beetles placed in cold storage survived to 2005 (240 of

282). A sub-sample of the beetles produced in 2004 showed a ratio of 1.1 males to 1.0 females (total of 111), with 35% of females gravid. Of the individuals shipped to the quarantine facility in June 2004, 38% survived for approximately 120 days and were placed in cold storage (18 of 48). However, none of these individuals survived to 2005.

Overall size was used to distinguish the smaller males from females, although some overlap was observed. No consistent differences were observed in the shape of the maxillary palps, coloration patterns (Waloff & Richards 1958), or the size and shape of tarsi/tarsal pads (J.H. Sun, personal communication), which have been used to distinguish males from females in other *Gonioctena* species. Upon dissection, differences between males and females were noted by the presence of ovaries or developing eggs in females (**Figures 1.1** & **1.2**).

Adult Kudzu Consumption and Time to Diapause: Insects reared on kudzu took 15.0 ± 0.4 days from the time they emerged as adults until they burrowed into the soil for diapause. During this period, single pairs of beetles consumed 10.5 ± 0.48 cm^2 (n = 6) of kudzu foliage per day. Individual beetle consumption was thus estimated to be 5.2 ± 0.24 cm^2 of kudzu foliage per day. When single beetles were fed on kudzu for 7 days, each insect consumed an average of 7.9 ± 0.42 cm^2 (n = 7) of foliage per day.

Adult No-Choice Tests: Over 90% of the adults given only kudzu survived to diapause, and all of these survived to 2005 (**Table 1.1**). No insects survived to diapause on joint-vetch, cowpea, or bush bean, while one individual given pole bean foliage survived to diapause but not to 2005. Half of the insects given hog-peanut survived to diapause and

to 2005, while 71% of insects given soybean survived to diapause, and 38% survived to 2005. Insects did not consume cowpea or joint-vetch foliage, and each insect ate an average of 1.1 cm^2 and 4.7 cm^2 of pole bean and bush bean, respectively. For kudzu, soybean and hog-peanut, insect damage was so great during each four-day interval that leaflets could not be recreated to determine leaf area eaten. Estimates of feeding damage, based on individual insect consumption of kudzu, are provided for comparative purposes. For those insects that did not survive to diapause, the average lifespan on pole bean, bush bean, joint-vetch, and cowpea was less than 15 days (**Table 1.1**).

Larval No-Choice Tests: Naïve larvae presented with bush bean, pole bean and cowpea were not able to complete larval development, and survival time was similar to that of insects given water only (**Table 1.2**). Development time was longest for larvae reared on soybean, intermediate for hog-peanut, and shortest for larvae reared on kudzu (**Table 1.3**). Larval survival was greater than 40% on these three plants. Survival to adulthood was greatest for insects reared on kudzu, but over 20% survived to adulthood on soybean, and 50% on hog-peanut. Leaf area consumption was the same for kudzu and soybean (not determined for hog-peanut; **Table 1.3**).

Larval Choice Tests: Naïve larvae ate more kudzu than soybean foliage when given a choice (**Table 1.4**). In this test the larval stage lasted 6.0 ± 0.00 days, with 57.1% of larvae surviving to pupation (8 of 14 insects). Adults emerged 9.0 ± 0.00 days later and adult emergence was 35.7% (5 of 14 insects). Development rates of insects that survived each stage were comparable to insects reared on kudzu.

Discussion:

Under quarantine conditions, *Gonioctena tredecimmaculata* exhibited (ovo)viviparous reproduction (Kudo et al. 1995), short development times (~ 16 d total), a long lifespan of reproducing adults (> 120 days), and high fecundity. In addition, insects reared as larvae in plastic tubs displayed high adult emergence rates (71%), and overwintering in cold storage yielded 85% survival. Size served as the best trait to separate the smaller males from larger females, although overlap was observed. The shape of the maxillary palps and color differences used to distinguish males from females in other *Gonioctena* species [*Phytodecta* (*Gonioctena) olivaceae*; Waloff & Richards 1958] were not reliable.

Based on our observations, the life cycle of *G. tredecimmaculata* in quarantine is similar to reports of other *Gonioctena* species. Eggs are laid and larvae emerge shortly after deposition to feed on leaf material. Fourth instars dig into the soil for pupation (Takizawa 1976), and adult insects emerge approximately 10 days later. *Gonioctena tredecimmaculata* then returns to host foliage for approximately two weeks to feed, followed by a return to the soil (without copulation or reproduction) as part of an obligate diapause [similar to *Phytodecta* (*Gonioctena*) *olivacea* (Forster), Waloff & Richards 1958]. Insects emerged from diapause the following year, mated, and reproduced continuously throughout the summer of 2005.

The life cycle of *G. tredecimmaculata* imposed two limitations on our study. First, it was not possible to determine the fecundity of females during the study period since insects were shipped directly from China in June of 2004, and could have reproduced before collection and shipment. Additionally, insects arriving to the

quarantine facility could have been a mixture of first and second year adults, making it difficult to determine the actual number of reproducing individuals, and consequently the average fecundity per mature female. Second, although adult and larval feeding occurred on kudzu, soybean and hog-peanut, the life cycle of *G. tredecimmaculata* prevented us from demonstrating that a naïve insect has the potential to develop and subsequently reproduce on these hosts. Because the adults produced each year do not reproduce until the following year, we are limited to saying that *G. tredecimmaculata* can consume kudzu, soybean and hog-peanut as both adults and larvae. However, the results of a companion study comparing insect response to field- and greenhouse-grown foliage (Chapter 2) demonstrate equal reproduction on kudzu and soybean by laboratory reared adults.

Despite the aforementioned limitations, we were able to evaluate the potential for *G. tredecimmaculata* to feed and develop on two plants of agricultural or intrinsic value. Soybean and American hog-peanut were also the two test plants that are most closely related to the target weed, as all three species are placed within the Phaseoleae subtribe Glycininae (Birdsall et al. 2004). Feeding on these species by *G. tredecimmaculata* is in accordance with Wapshere's (1974) phylogenetic approach to host plant testing, which states that species closely related to the target weed are at a greater risk of attack than distantly related species.

During a larval no-choice experiment, development times were significantly different for kudzu and soybean, and insects took longer to develop on soybean by approximately 2 days. For insects reared on hog-peanut, the duration of the larval period

was significantly different from both kudzu and soybean, and values for both the larval period and time as pupae were intermediate to those of kudzu and soybean. Leaf area consumption by larvae in the no-choice test was the same for kudzu and soybean, but was not determined for hog-peanut. In a larval choice test between kudzu and soybean, larvae consumed significantly more kudzu, and development times were similar to those of insects fed kudzu only. The total amount of foliage consumed in all experiments (choice and no-choice) was approximately 15.5 cm^2 for each larva that survived to diapause, regardless of the host foliage consumed. This suggests that a certain amount of foliage must be consumed for insects to complete larval development, though this hypothesis was not tested. In terms of adult insects, percent survivals to diapause and to 2005 were highest for insects kept on kudzu, soybean or hog-peanut. Taken together, these results suggest that *G. tredecimmaculata* is not a specialist feeder on kudzu, although this might be the insect's preferred host.

In China, *G. tredecimmaculata* is not identified as a pest of soybean or other plant species (Kogan & Turnipseed 1987, Talekar 1987). However, our results demonstrate that soybean is within the physiological host range of the insect, since larvae completed their development and adults survived to the following year. Therefore, it is possible that behavioral barriers to feeding and oviposition limit the impact of *G. tredecimmaculata* on soybean in China. To test this hypothesis, a field experiment in which kudzu and soybean are grown in close proximity to each other is underway in China. Surveys will determine the use of soybean as a host plant by *G. tredecimmaculata*, and potentially investigate the extent of feeding damage imposed on the plant.

In the assessment of a potential biological control agent, the rule is to not release an insect capable of completing development on a crop or desirable plant (Harris 1988). Therefore, even if soybean is not found to be within the ecological host range of *G. tredecimmaculata* in Asia, the ability of this species to consume both soybean and the native *Amphicarpaea bracteata* (hog-peanut) in quarantine probably precludes its further consideration as a biological control agent of kudzu.

Figure 1.1. Gravid female with elytra and cuticle removed to expose developing larvae

Figure 1.2. Male beetle with elytra and cuticle removed to expose fat body covering testes

Table 1.1. Percent survival, leaf area consumption, and time until death for non-survivors in an adult no-choice host specificity test

Host Plant	N [a]	Percent Survival to Diapause	Percent Survival to 2005	Area Consumed per Individual ($\overline{X}$ cm^2)	Time Until Death for Non-Survivors ($\overline{X}$ days ± SEM) (N) [b]
Pueraria lobata (kudzu)	12	91.7	91.7	75 *	18.0 (1) a
Glycine max (soybean)	24	75.0	37.5	71 *	21.3 ± 8.19 (6) a
Amphicarpaea bracteata (hog-peanut)	6	50.0	50.0	68 *	3.0 (1) a
Phaseolus vulgaris (pole bean)	12	16.7	0	1.1	14.7 ± 7.58 (11) a
Phaseolus vulgaris (bush bean)	12	0	0	4.7	14.7 ± 1.93 (12) a
Aeschynomene virginica (jointvetch)	12	0	0	0	4.0 (12) a
Vigna luteola (cowpea)	12	0	0	0	4.0 (12) a
Water only	12	0	0	NA	4.5 ± 0.15 (12) a
F					2.48
df					7, 59
P					0.0264

Means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)
*, Estimated (entire leaves consumed); NA, Not Applicable.
[a] Number of insects on a given host plant; insects kept in groups of 6, with foliage changed every 4 days
[b] (N) represents number of insects that did not survive to diapause

Table 1.2. Time until death for naïve larvae that did not survive in a no-choice host specificity test

Host Plant	N	Time Until Death ($\overline{X}$ days ± SEM)
Phaseolus vulgaris (bush bean)	7	2.4 ± 0.20 a
Phaseolus vulgaris (pole bean)	6	2.0 ± 0.00 a
Vigna luteola (cowpea)	6	2.3 ± 0.21 a
Water only	8	1.8 ± 0.31 a
F		1.90
df		3, 23
P		0.1577

Means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)

Table 1.3. Development of insects in a larval no-choice host specificity test

Host Plant	N	Larval Survival (N) [a]	Larval Period ($\overline{X}$ days ± SEM) [a]	Area Consumed ($\overline{X}$ cm^2 ± SEM)	Time as Pupae ($\overline{X}$ days ± SEM) [b]	Survival to Adulthood (N)
G. max (soybean)	14	42.9% (6)	7.5 ± 0.34 a	14.8 ± 1.03 a	9.3 ± 0.33 b	21.4% (3)
A. bracteata (hog-peanut)	8	50.0% (4)	6.0 ± 0.00 b	*	10.0 ± 0.00 ab	50.0% (4)
P. lobata (kudzu)	14	85.7% (12)	5.8 ± 0.11 c	16.3 ± 0.63 a	10.2 ± 0.15 a	64.3% (9)
F			21.34	1.66	5.20	
df			2, 19	1, 16	2, 13	
P			< 0.0001	0.2160	0.0219	

In a column, means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)
*, All available foliage consumed
[a] For survivors to pupation
[b] For adults that emerged from pupation

Table 1.4. Leaf area consumption by naïve larvae in a choice test with kudzu and soybean

Host Plant	Area Consumed ($\overline{X}$ cm^2 ± SEM)	T-Value
Pueraria lobata (kudzu)	14.0 ± 1.52	3.95 (P = 0.0055)
Glycine max (soybean)	2.5 ± 1.49	

N = 8 (survivors to pupation); T-Value of a Paired t Test computed using the difference between feeding values (kudzu area eaten – soybean area eaten)

Chapter 2 – Host plant phenology and host specificity of a potential biological control agent: a comparison of field- and greenhouse-grown foliage

Introduction:

In June of 2004, *Gonioctena tredecimmaculata* (Jacoby) (Coleoptera: Chrysomelidae), a leaf-feeding beetle from China, was sent to a U.S. quarantine facility to determine its suitability as a biological control agent for kudzu. No-choice host specificity tests indicated that this insect will consume tender, young foliage of soybean [*Glycine max* (L.) Merr.] and American hog-peanut [*Amphicarpaea bracteata* (L.) Fernald] in addition to kudzu (Chapter 1). However, the studies on soybean were conducted with greenhouse grown potted plants, which look quite different from field-grown plants. Because this insect is not known to be a pest of soybean in China (Kogan & Turnipseed 1987, Talekar 1987), the possibility remained that *G. tredecimmaculata* might reject field-grown soybean despite feeding on greenhouse foliage.

It is well known that interactions between insects and plants are mediated by a number of factors that include, but are not limited to, insect searching behavior, host plant phenology, insect feeding behavior, plant resistance mechanisms, and palatability/digestibility of host plant material (Bell & Cardé 1984). However, in the arena of host specificity testing for a biological control agent, many of these factors are eliminated in experiments conducted in quarantine. While this may be beneficial in terms of evaluating the physiological host range of an insect, certain factors of plant-insect

interactions that are omitted in quarantine studies can influence host selection and acceptance by the insect (Blossey 1995), and potentially the validity of specificity trials. Therefore, a major aspect of this study was to investigate the potential for differences in foliar traits, as mediated by the growing environment of a plant, to influence insect response and specificity in quarantine.

Previous work has suggested that field plants differ from greenhouse plants based on exposure to environmental stresses and attack by herbivores, predators and pathogens in the field (Underwood et al. 2002). For example, induction by wind (Cipollini 1997), herbivore feeding (Pullin & Gilbert 1989, Baur et al. 1991, Kogan & Fischer 1991, Maruicio & Rausher 1997), high temperatures (Wellso & Hoxie 1982), low soil moisture levels (Wellso & Hoxie 1982, Jenkins et al. 1997) and mycorrhizal colonization (Gehring & Whitham 1994) can increase mechanical or chemical resistance traits in field plants. Differences between field and greenhouse plants are also attributed to variation in light levels when plants are grown under glass. For instance, the phenolic content of *Sorghum bicolor* (L.) Moench is known to vary with increasing light intensity, and is higher for field versus laboratory plants (Woodhead 1981).

Variation in the expression of mechanical or chemical resistance factors can lead to differential feeding, survival, and fitness of herbivores presented with field- or greenhouse-grown plants. Field plants, for example, had tougher leaves and more phytochemically derived phenolic dimers than greenhouse maize plants (*Zea mays* L.), which reduced foliage consumption by the European corn borer [*Ostrinia nubilalis* (Hübner)] (Bergvinson et al. 1995). Increased trichome densities, which are typically

found on young foliage compared to older foliage (Johnson 1975) and induced by a number of environmental factors, are associated with decreased feeding, oviposition and insect larval nutrition (Levin 1973). Trichome length can also influence insect biology. For example, wheat plants (*Triticum aestivum* L.) with longer trichomes had fewer cereal leaf beetle [*Oulema melanopus* (L.)] eggs and reduced larval survival compared to plants with shorter trichomes (Hoxie et al. 1975). Insect response to trichomes is case dependent, however, since preferred leaves may be those that are densely pubescent (Kendall et al. 1996), and in some cases presence or absence alone may dictate preferences (Gannon & Bach 1996).

Leaf toughness, another trait that can vary between field- and greenhouse-grown foliage, plays a defensive role against insect herbivores. Tougher leaves of *Salix* spp. eroded the cutting surface of the imported willow leaf beetle's (*Plagiodera versicolora* Laich) mandibles, causing insects to eat more slowly (Raupp 1985). Reduced feeding capacity may have consequences for insect reproductive success, since fecundity is correlated with adult consumption in *P. versicolora* (Raupp 1985). Insect feeding was also reduced on leaves of female *Baccharis halimifolia* L. plants when compared to male plants of the same species. Female plants had leaves that were tougher than male plants, although nutritional differences did not exist between the sexes (Krischik & Denno 1990). In soybean, irradiance levels alter both leaf toughness (Lugg & Sinclair 1980) and thickness (Van Volkenburgh & Davies 1977), and higher values for field versus greenhouse plants (Lugg & Sinclair 1980) might influence insect feeding behavior.

Nutritional factors, such as the total carbon (C) and nitrogen (N) contents of leaves and the C:N ratio, can vary in plants as a result of light levels, light quality (Letourneau 1997), and the method of nitrogen acquisition from the environment (Harris et al. 1985, Harper 1987). Both elements are primary plant resources that can either be limiting in a plant or allocated to defense. In the latter situation, carbon and nitrogen are directly involved in the construction of secondary compounds or protective structures (Mooney et al. 1983). For insects, nitrogen is a critical limiting nutrient (Mattson 1980) and is thus positively correlated with herbivory (Xiang & Chen 2004). For example, high inputs of nitrogen for agricultural plants can lead to greater rates of feeding and oviposition by insect herbivores (Letourneau 1997). The ability of insects to utilize or assimilate nitrogen, however, can be influenced by leaf water content, and low levels of leaf water can also slow the development of larval Lepidoptera (Scriber 1977). Therefore, insect development might be limited more by water and nitrogen than nitrogen alone (Scriber 1977, Scriber & Feeny 1979).

Plant chemistry is another important character of plant-insect interactions, which can alter insect acceptance and utilization of host material. Assays quantifying total phenolic and tannin contents of leaves, for example, successfully explain feeding preferences of certain herbivores (Waterman & Mole 1994, but see Berenbaum & Zangerl 1992, Heil et al. 2002). In general, phenolic content of plants is positively correlated with light intensity (Woodhead 1981, Dudt & Shure 1994, Waterman & Mole 1994) and negatively correlated with nitrogen supply (Waterman & Mole 1994). Therefore, field plants are expected to have increased quantities of phenolic compounds

compared to greenhouse plants, making the less defended greenhouse plants more acceptable to herbivores.

Based on the idea that extrinsic factors (biotic and abiotic) can induce plant mechanical or chemical defenses, that these resistance mechanisms can therefore differ in their expression between the field and greenhouse, and that these variations can influence insect herbivore biology, an important goal of the current study was to determine how variation in plant foliar traits might impact the response and specificity of a potential biological control agent in quarantine studies. The leaf traits investigated in this study represent both external and internal features of host foliage as perceived by an insect following host location. These include trichome density and length, leaf toughness, water content, total carbon and nitrogen contents, and total phenolic content. Foliar traits were examined in conjunction with feeding and oviposition preference, as well as reproduction by *Gonioctena tredecimmaculata*. The results of this investigation are critical for a complete understanding of the biology of *G. tredecimmaculata* and the potential for this insect to serve as a biological control agent of kudzu. Comparisons between field- and greenhouse-grown foliage will establish whether *G. tredecimmaculata* is able to complete its life cycle on an important agricultural crop, soybean, and elucidate factors that may impact successful host specificity testing in quarantine facilities.

Materials and Methods:

Test Plants

Soybean: Seeds of soybean [*Glycine max* (L.) Merr.; RT- 3940 N] were purchased from Southern States (Newark, DE) and used for plantings in both the field and greenhouse to

maintain genetic homogeneity. Seeds were pretreated with Rival and Allegiance – FL to reduce the impact of soil organisms in the field, and soybean plants were Roundup Ready. These treatments are common for soybean grown in Delaware (Jon Hummel, personal communication).

In the field, soybean was planted with the following specifications: 76 cm row width, 6 plants per 30.5 cm, and 150,000 seeds per 0.4 hectare on clean tilled land (Bruce Vasilas, personal communication). Plantings were made on two separate dates with the intent to utilize plants of the V2 growth stage throughout the summer, as differences in plant age might affect insect growth and development (Hart et al. 1983), as well as phonological plant traits (Johnson 1975). The V2 growth stage is characterized by the presence of unfolded, fully developed trifoliate leaves at two nodes along the stem (Vasilas et al. 1991). The first 0.4 hectare was planted on 4 May 2005 and the second on 13 May 2005. Both plots were treated with Roundup on 25 May 2005 to reduce weed populations within the field.

At approximately 27 °C during the day and 20 °C at night, soybean plants in the greenhouse grew about twice as fast as field plants. Due to this rapid growth rate, plantings were made every 7 days between 6 May 05 and 17 August 05, to insure a steady supply of V2 plants. For each planting event, 165 – 180 square pots (10.2 cm) were filled with Pro-mix (Premier Horticulture, Red Hill, PA) and two seeds were sown at a time. When seedlings reached the V1 stage, one plant was removed by cutting to reduce resource competition within a pot. Plants were hand watered twice daily, fertilized biweekly, and grown under available natural light.

Kudzu: In order to maintain genetic homogeneity, seedpods were collected within a single field site in Fair Hill, Maryland (39° 42' 14.83" N, 75° 47' 59.39" W) on 23 February 2005. Pods were hulled and seeds were stored at room temperature. Kudzu exhibits seed dormancy as a function of a hard seed coat, and seeds were boiled for 10 seconds in distilled water in order to overcome this dormancy (Susko et al. 2001). Once boiled, seeds were placed on a tray with a moist paper towel, and plastic wrap covered the tray to retain moisture. Seeds were planted the next day in round pots (15.2 cm diameter) containing Pro-mix, and a total of 5 seeds were dispersed and sown 2 cm into the soil in each pot (Susko et al. 1999). Between two planting dates (5 May and 20 May 2005), 900 pots were planted with 3 - 5 seeds per pot.

Plant Characteristics

Similar criteria were used to select host foliage for all experiments, including investigations into leaf traits and insect physiology. An attempt was made to collect the tender, young leaves preferred by *G. tredecimmaculata*. For soybean, the two unfolded trifoliate leaves at the apical end of a V2 plant were collected from field and greenhouse plants. Later in the season when field plants were older than the V2 stage, however, the two most apical unfolded leaves were collected. Trifoliate leaves were never collected from reproductive soybean plants. For kudzu, field leaves were those that were lighter green and near the end of a terminal. Each leaflet was approximately 8 – 10 cm long. In the greenhouse, leaves collected from kudzu were light green and leaflets were approximately half the size of field-collected leaflets.

Leaves were collected from plants with pruning sheers and were placed in a self-sealing plastic bag after cutting at the petiole. For soybean, the top leaf was cut first, followed by the one below it. After 2 leaves had been removed from a plant (excluding field kudzu), the plant was cut at the base and discarded. For each collecting event, when all the leaves of a foliage type had been collected and placed in a single bag, these leaves were transported in a cooler containing an ice pack to keep foliage from wilting or desiccating.

Experiments with emphasis on an isolated leaf part focused on the same region of a leaflet. Previous work has shown that the mid-region of a leaf represents the best estimate of trichome density and length (Wellso & Hoxie 1982), and normal feeding by *G. tredecimmaculata* adults and larvae occur there. Specifically, measurements of trichome density, length, and leaf toughness were made half way from the mid-vein to the leaf edge, and half way from the top to the bottom of a leaflet.

Plant characteristics analyzed in this study are reported from external to internal features of the leaf. Although analysis of these traits did not occur in the specified order, it is suspected that their influence on the insects proceeds from external to internal once the insect has located its host.

Trichome Density: As mentioned above, only the young, newly expanded leaves of kudzu and soybean were examined, since these are the leaves consumed by *G. tredecimmaculata*. It is anticipated that newly expanded leaves on a plant have similar densities of trichomes throughout the life of that plant, since physical defenses play an important role early on, giving way to chemical defenses in older foliage (Johnson 1975).

Six trifoliate leaves of each foliage type were collected randomly and brought to the laboratory on 2 August 2005 to determine trichome density per mm^2. Leaflets are the feeding unit of the beetle, and these were removed from leaves by cutting at the petiolule and placed on a table. Twelve leaflets were randomly selected for analysis under magnification, and an external light source was manipulated to illuminate trichomes by placing it parallel to the base of the microscope. Digital images of the adaxial surface were taken using a Leica DC 100 camera (Leica Microsystems Inc., Bannockburn, IL) and viewed in Photoshop 7.0.1 (Adobe Systems Inc., San Jose, CA) under low and high magnification (field size of 180 mm^2 and 7 mm^2, respectively). Images were printed in black and white on regular computer paper and trichomes were counted with a hand counter. To correct for differences in the field size, trichome densities were divided by the magnification to yield trichomes per mm^2.

Trichome Length: In order to determine trichome length, 10 trichomes per image were randomly selected and measured with a ruler (a total of 120 trichomes were measured per foliage type). To correct for differences in the size of the image, a ratio was established between the field size of the hard copy image and the field size of the image on the computer screen (both in mm). When this value was multiplied by the measurement of a trichome on the hard copy, the result was the measured length of the trichome on the computer. This value was then multiplied by the ratio between the actual field size and the field size of the computer image (both in mm). These calculations returned the actual length of the trichome, which was averaged for each plant. Results are an average of the average trichome length for each host plant.

Leaf Toughness: A TA.XT2i Texture Analyzer (Texture Technologies, Scarsdale, NY) in the Department of Animal and Food Sciences at the University of Delaware was used to measure leaf toughness on 24 June 2005. This machine reports toughness as the amount of force (in grams) necessary to puncture a hole in an object. Fifteen trifoliate leaves of each foliage type were collected and two points were measured and averaged for two of the three leaflets per leaf (a total of 30 analyzed leaflets). A 2.0 mm flat tip probe was selected for analysis (as in Kudo 2003), and the following specifications were made: pretest probe speed was 2.0 mm/s, and the test speed was 1.5 mm/s until the sample ruptured. Leaflets were held down with a 70.5 g washer with a 3 cm opening in the middle. This added resistance and prevented the leaf from collapsing around the probe.

Leaf Water Content: Ten trifoliate leaves of each foliage type were collected and brought to the laboratory on 17 June 2005. Leaflets were removed from leaves as described above, and the three leaflets were weighed together to determine the fresh weight of each leaf. Each set of leaflets was placed in a labeled paper bag, which was dried in a drying oven (Model 5015-54 Lab Oven; Cole Parmer Instrument Co., Chicago, IL) for 6 hours at 64 °C. On 20 June 2005, leaflets were removed from the bag and the dry weight of each leaf was determined. Percent water content was the dry weight divided by the fresh weight.

Total C and Total N: Approximately 30 trifoliate leaves of each foliage type were collected on 18 June 2005 and transported to the laboratory. Leaflets were removed as above, and all leaflets of a foliage type were placed in a single paper bag. Samples were

dried in a drying oven for 6 hours at 64 °C. Dried leaf material was ground into a fine powder using a mortar and pestle and was mixed thoroughly for homogeneity. Rinses of the mortar and pestle with distilled water were completed between foliage types to prevent contamination by the previous sample.

Powdered samples were delivered to the Soil Testing Laboratory of the Plant and Soil Sciences department at the University of Delaware in sterile, 50 ml VWR plastic vials. Ten samples of 10 mg each were processed with an Elementar Vario-Max CN analyzer (Elementar America, Inc., Mt. Laurel, NJ). Samples were analyzed by combustion using the Dumas Technique, and results are reported as the percent composition of each element by weight in the sample (percent weight per 10 mg).

Total Phenolic Content: A protocol developed by Fathi Halaweish (South Dakota State University, Brookings, SD) was modified and used to determine the total phenolic content of kudzu and soybean foliage. For this procedure, approximately 30 trifoliate leaves of each foliage type were collected on 29 June 2005, transported to the laboratory, and prepared as powdered samples (see above). Extraction of 0.25 g of dried leaf material was accomplished in 10.0 ml of 99.9% HPLC grade methanol (Fisher Scientific Company L.L.C., Pittsburgh, PA). The sample was homogenized in a 50 ml test tube using a Ryobi 3/8" Variable Speed Drill D40 (Techtronic Industries Co. Ltd., Tsuen Wan, New Territories, China) and a Teflon tipped pestle for approximately 3 minutes. The test tube was immersed in a water/ice bath during this process to prevent the buildup of heat. Extracted samples were then centrifuged for 10 minutes at 4000 rpm [IECCentra-4B Centrifuge; International Equipment Company (Division of Damon),

Needham Heights, MA]. The supernatant was transferred to a 25 ml volumetric flask (Fisher Scientific) and brought up to mark with methanol.

Once samples were prepared, 500 µl were combined with 3050 µl distilled H_2O and 150 µl Folin-Denis reagent (Fisher Scientific) and vortexed. Next, 300 µl of 35% Na_2CO_3 were added and the sample was vortexed again. Absorbance was noted after 15 minutes at 730 nm in a diode array spectrophotometer (Agilent 8453; Agilent Technologies, Inc., Palo Alto, CA). The concentration of phenolics was determined by comparing sample absorbance at 730 nm to the absorbance of known concentrations of tannic acid (Sigma-Aldrich Corporation, St. Louis, MO) standards diluted with methanol (0.2, 0.4, 0.6, 0.8 mg / ml) using UV-Visible ChemStation Software (Agilent Technologies, Inc.). The blank created for spectrophotometric analysis contained all reagents excluding sample extract.

Insects

Insects used for experiments in 2005 were reared on kudzu in 2004 and overwintered in sphagnum moss in a 1.7 °C chamber. Insects were removed from cold storage and placed in a 13 °C chamber for 14 days, then moved to rearing conditions of 25.6 ± 0.02 °C, 16L : 8D. Insects were individually removed from sphagnum moss and placed in a plastic tub (17 X 12 X 6 cm; Pioneer Plastics Inc., Dixon, KY), where mating was observed. Size differences were used in an attempt to separate the smaller males from females after all insects had been recovered. However, this character is not entirely accurate, as the species is said to exhibit "no distinct sexual dimorphism" (Bezděk 2002). Nevertheless, suspected mating pairs of adults were placed into plastic tubs containing 5 cm sterilized

Pro-mix as substrate and two trifoliate leaves. This setup was replicated 30 times for each host plant (kudzu or soybean) and foliage type (field or greenhouse; a total of 120 plastic tubs) and was used to examine insect fecundity, percent reproduction, and female oviposition preference.

Fecundity and Percent Reproduction: Insects in plastic tubs were checked daily from 18 June to 30 August 2005 to investigate feeding and reproductive behavior. Tubs in which foliage had feeding damage were recorded, and larvae were collected, counted, and used for other experiments. Foliage was changed or added every other day to ensure constant foliage quality: two trifoliate leaves were added on day one, one trifoliate leaf was added on day three, and on the fifth day all old foliage was removed and two new trifoliate leaves were added.

Paired insects from different cages of the same treatment were combined on 12 July in an attempt to increase the number of reproducing adults. Following this period, two mating pairs were contained within each replicate, for a total of 4 insects per tub.

Oviposition Choice: Ten plastic tubs with a mating pair of insects from each treatment were used to test the oviposition preference of adult females. This test was conducted on 10 August 2005 and presented adult beetles with a choice between field and greenhouse foliage of the same host plant (kudzu or soybean). One trifoliate leaf from the field and one from the greenhouse were placed inside the plastic tub and touched in the middle. Insects were placed on the soil in the middle of the plastic tub and were allowed to feed

and reproduce for 24 hours. After this time, adult feeding and the location of larvae were recorded.

Larval Development: Larvae collected during the fecundity and percent reproduction experiment were transferred directly to a Petri dish (10 X 1.5 cm) lined with filter paper. In most cases, larvae were given the same host plant and foliage type as the adults that had produced them. However, when numbers of larvae from a given host plant and foliage type were too low, excess larvae from other treatments were collected and utilized. When this was done, un-hatched or newly hatched, teneral larvae from the same host plant but different foliage type (field or greenhouse) were used. Filter paper was kept moist with distilled water, and foliage was changed every other day. Insect development was monitored daily by recording the presence of head capsules. When fourth instars began wandering, they were transported to 163 ml soufflé cups (Solo Cup Company, Highland Park, IL) with Pro-mix for pupation. Pupation cups were sprayed with distilled water to maintain soil moisture and protect pupating insects from desiccation. Time as pupae was recorded from the time larvae were added to the soufflé cups until adult emergence. Upon emergence, adult weight was recorded in milligrams.

'Naïve' Adult Choice (Field vs. Greenhouse): Newly emerged adults were placed upside-down in the center of a Petri dish (10 X 1.5 cm) and presented with a choice of 3 field and 3 greenhouse leaf disks (1.1 cm diameter cork borer # 5; Carolina Biological Supply, Burlington, NC) for 21 hours. Leaf disks were placed with the adaxial surface facing up, arranged in an alternating pattern, and were evenly spaced on moistened filter paper

within the dish. At the conclusion of the experiment, all leaf disks were taped onto a sheet of white paper with clear tape, photocopied, and scanned into the computer (HP Scanjet 3970; Hewlett-Packard Company, Palo Alto, CA). Leaf area analysis used ImageJ Software (ImageJ 1.36 for Windows; National Institutes of Health, Bethesda, MD) to compare the leaf area remaining after insect feeding to a set of 6 leaf disks kept under the same conditions but not exposed to insects. For example, the leaf area of 3 field foliage disks after insect feeding was subtracted from the leaf area of 3 undamaged leaf disks set up at the same time to return the amount of leaf area consumed.

Adult No-Choice (Kudzu vs. Soybean): Adult insects previously kept on one host plant (kudzu or soybean) for approximately 60 days were placed in the center of a Petri dish (10 X 1.5 cm) and presented with 6 leaf disks (same size as above) of either greenhouse soybean or field kudzu for 17.5 hours. Thus, insects kept on soybean were given either kudzu or soybean, and adults kept on kudzu were given either kudzu or soybean. These disks were placed with the adaxial surface facing up and were evenly spaced on moistened filter paper within the dish. Leaf area analysis was conducted using the same method as above, with a set of 6 undamaged leaf disks used to determine insect foliage consumption.

Adult Choice (Kudzu vs. Soybean): Adult insects previously kept on one host plant (kudzu or soybean) for approximately 60 days were placed upside-down in the center of a Petri dish (10 X 1.5 cm). Insects were presented with 3 leaf disks (same size as above) of both field kudzu and greenhouse soybean for 18.5 hours. These disks were placed with

the adaxial surface facing up, arranged in an alternating pattern, and were evenly spaced on moistened filter paper within the dish. Leaf area analysis was conducted using the same method as above, utilizing a set of 6 undamaged leaf disks to determine insect foliage consumption per host plant.

Data Analysis

The SAS System (Version 9.1; SAS Institute Inc., Cary, NC) was used to perform all statistical procedures. Trichome density and length, leaf toughness, water content, total nitrogen and total carbon contents, total phenolic content, larval development, duration of the pupal stage, adult weight, and adult no-choice data were analyzed with ANOVA followed by Tukey's Studentized Range (HSD) Test to evaluate differences between host plants and treatments. T Tests were also used for comparisons between foliage types within a host plant for all the aforementioned traits. Paired t Tests were used to examine feeding in the adult choice tests comparing field to greenhouse foliage and kudzu to soybean foliage. This test assumes that observations are not independent of each other and that selection to feed and not feed is coupled. Data from percent water content and total nitrogen content determinations were arcsine transformed, as percentages fell outside the range of 30 – 70% (Snedecor & Cochran 1967).

Results:

Test Plants

Germination of soybean was high in the field and greenhouse, with nearly all seeds producing plants. Field kudzu foliage was abundant for collection. However,

greenhouse kudzu germination was low, with 33.2% (299 of 900) of pots sown with approximately 5 seeds per pot having 1 or more kudzu plants.

Plant Characteristics

Trichome Density: For both soybean and kudzu, field foliage had twice as many trichomes per mm^2 as greenhouse foliage (**Table 2.1**). Average trichome densities for field and greenhouse plants were each 17.5 times greater on kudzu than soybean.

Trichome Length: For each host plant, trichome lengths were significantly different between field and greenhouse foliage (**Table 2.1**). Greenhouse-grown soybean foliage had longer trichomes than field grown foliage ($t = -3.55$, $P < 0.005$), and the reverse relationship was true for kudzu ($t = 3.13$, $P < 0.005$).

Leaf Toughness: Leaves from the field were approximately 1.5 times tougher than leaves from the greenhouse for both kudzu and soybean (**Table 2.2**). Toughness of field-grown soybean and kudzu was similar, as was toughness of greenhouse-grown soybean and kudzu.

Leaf Water Content: Overall, greenhouse kudzu had the highest water content, which differed from field and greenhouse soybean, as well as from field kudzu foliage (**Table 2.3**). For both kudzu and soybean, however, field foliage had significantly lower water contents than greenhouse foliage when compared within host plant using t tests.

Total C and Total N: Percent composition of carbon was significantly higher in field foliage compared to greenhouse foliage for both kudzu and soybean. Soybean foliage

had significantly more carbon by weight than kudzu when both plants were grown in the greenhouse (**Table 2.4**), but both hosts had similar carbon compositions when plants were grown in the field.

Nitrogen content was significantly different for all treatments, with no consistent relationship between host plant or foliage type (**Table 2.4**). Greenhouse soybean had the highest nitrogen content, while greenhouse kudzu had the lowest nitrogen content of all treatments.

Total Phenolic Content: Total phenolic content was not significantly different for any treatment overall (**Table 2.5**). However, when analyzed individually, field soybean had a significantly higher phenolic content then greenhouse soybean.

Insects

Fecundity and Percent Reproduction: For each host plant and foliage type, fewer than 9.0% of adults reproduced in the first 26 days (**Table 2.6**). After combining insects, average fecundity and percent reproduction increased in the next 23 days (**Table 2.7**). For the entire the summer, percent reproduction was low, less than half of the tubs contained reproducing adults, and larval production was highly variable for all treatments. There were no significant differences in the fecundity of adult insects when comparing host plant (kudzu and soybean) or foliage type (field and greenhouse).

Oviposition Choice: Sample sizes for each treatment were low (field soybean = 3, greenhouse soybean = 2, field kudzu = 1, greenhouse kudzu = 1) because so few of the adult pairs reproduced. When given a choice between field and greenhouse foliage, adult

beetles kept on field soybean foliage fed and deposited offspring on both field (5 larvae) and greenhouse (11 larvae) foliage. Adult beetles kept on greenhouse soybean foliage fed only on greenhouse foliage, but deposited offspring on both field (4 larvae) and greenhouse (5 larvae) foliage. Adult beetles kept on field kudzu foliage fed only on field foliage, but deposited offspring on both field (2 larvae) and greenhouse (1 larva) foliage. Adult beetles kept on greenhouse kudzu foliage fed on both foliage types and did not reproduce.

Larval Development: Larval development was one day longer on soybean than kudzu when foliage was from the greenhouse (**Table 2.8**). Overall, however, larval survival to adulthood was higher for insects reared on soybean when compared to kudzu. No difference was found for the duration of the pupal stage, and adult weight at emergence was similar for all insects that survived to adulthood. Development was significantly faster for insects reared on greenhouse compared to field kudzu foliage (**Table 2.8**).

'Naïve' Adult Choice (Field vs. Greenhouse): Newly emerged adult insects showed no preference for field or greenhouse foliage when given a choice between the two (**Table 2.9**). This response was the same for insects reared on both kudzu and soybean, although insects ate nearly twice as much kudzu during the experiment.

Adult No-Choice (Kudzu vs. Soybean): Insects kept on one host for 60 days showed no difference in feeding habits when given 6 leaf disks of the same or a new host plant (**Table 2.10**). Insects kept on soybean for 60 days showed no preference for kudzu or

soybean [T Value = 0.09, P = 0.9291, df = 18], but insects kept on kudzu for 60 days tended to consume more kudzu than soybean [T Value = 1.87, P = 0.0780, df = 18].

Adult Choice (Kudzu vs. Soybean): Insects reared on one host plant for 60 days and subsequently presented with 3 leaf disks of both host plants showed no difference in their feeding preference (**Table 2.11**). There was a trend for insects to consume more kudzu than soybean foliage despite the original host plant ($0.05 < P < 0.10$).

Discussion:

Overall, field foliage of kudzu and soybean exhibited greater leaf toughness, higher total carbon content, higher trichome density per mm^2, and reduced water content than greenhouse foliage. Field soybean foliage had a higher total phenolic content than greenhouse soybean foliage, and no differences were recorded for kudzu. Significant differences were detected between trichome length and total nitrogen content for field and greenhouse foliage, but no overall patterns in field versus greenhouse or kudzu versus soybean comparisons were identified for these traits.

In terms of plant characteristics, trichome densities in this study were conserved across treatments. Field foliage of both plants had twice as many trichomes per mm^2 as greenhouse foliage, and kudzu plants had 17 times the number of trichomes per mm^2 compared to soybean plants from the same growing condition. This relationship might indicate that trichome density is under genetic control in kudzu and soybean, with factors in the environment such as wind (Cipollini 1997) herbivore feeding (Baur et al. 1991), temperature and moisture level (Wellso & Hoxie 1982) regulating gene expression in the

field. Despite documented variation in the size, density, color (Lersten & Carlson 1987) and types of trichomes [glandular and non-glandular (Franceschi & Giaquinta 1983)] for soybean varieties commonly grown in the U.S., observed densities were comparable to a recent study by Styrsky et al. (2006). In addition, only non-glandular trichomes were observed, which is expected for fully expanded leaves (Franceschi & Giaquinta 1983).

These findings suggest that herbivore utilization of host plant material might differ for field and greenhouse foliage, since the structure of the plant surface (Städler 1986) and the presence or absence of secondary plant substances (Fraenkel 1969) are used in host selection. The leaf surface also functions as an assessment of the nutritional value of a plant, and is used to orient females to oviposit (Städler 1986). This latter point becomes increasingly important when larval development is rapid (as is true for *G. tredecimmaculata)*, since oviposition choice by females determines more closely the food resource encountered by first-instar larvae (Raupp & Denno 1983). However, despite the potential importance that differences in the surface structure and chemical composition of leaves can have on herbivore populations, no obvious disparities in insect feeding, ovipostion choice and reproduction were recorded.

In this experiment, attempts were made to control for plant age in the field and greenhouse, with the exception of leaf material from mature kudzu plants in the field. This goal was complicated by poor kudzu germination in the greenhouse, with only 33.2% (299) of 900 pots having one or more kudzu plants. While a number of factors may have contributed to this poor germination rate [over watering (Susko et al. 1999), planting depth (Susko et al. 1999), growth medium, dormancy breaking technique (Susko

et al. 2001), etc.], low numbers of plants imposed another variable between field and greenhouse kudzu foliage, such that more than two trifoliate leaves were utilized from healthy greenhouse plants before they were discarded. Further complications included infestation of greenhouse kudzu with two-spotted spider mites (*Tetranychus urticae* Koch) and black bean aphids (*Aphis fabae* Scopoli) near the middle of the summer. These infestations produced plants that were deemed unsuitable for host specificity testing, based on observable decreases in foliage quality (chlorosis and stippling). In the field, soybean plants were also infested midsummer, with adult Japanese beetles (*Popillia japonica* Newman) causing significant foliar damage. Despite this infestation, undamaged leaves were available for collection and use in trials, although induced defenses as a product of insect herbivory might have influenced insect feeding and survival (Hart et al. 1983, Chiang et al. 1987, Kogan & Fischer 1991, Underwood 2000). This is case for the Mexican bean beetle (*Epilachna varivestis* Mulsant), such that larvae fed on soybean foliage previously damaged by the soybean looper [*Pseudoplusia includens* (Walker)] exhibited longer development times and lower final weights than larvae that fed on undamaged foliage (Lin & Kogan 1990).

In some systems, total phenolic assays have been used to explain the feeding preference of an herbivore (Waterman & Mole 1994), although these tests are subject to criticism and do not always produce reliable results (Berenbaum & Zangerl 1992, Heil et al. 2002). One criticism is that these assays fail to identify the specific compounds involved in defense against a particular herbivore (Heil et al. 2002). This has important consequences, as the biological activity of phenolic compounds can vary with chemical

structure and the target organism (Heil et al. 2002). Furthermore, differences in sample preparation techniques can lead to wide variations in the analytical results obtained (Luthria 2006). In the current study, the method of extraction produced significant differences in the total phenolic content of leaves within a host plant and foliage type (data not shown). Attempts to overcome this variability by using another extraction technique were unsuccessful, and future work is needed to develop a protocol yielding consistent results. Therefore, limited conclusions can be made regarding the total phenolic content of leaf material analyzed in this study based on low sample numbers.

Larval development times and insect survival values recorded in this study did not meet my predictions. Development was no different on field kudzu than on field- or greenhouse-grown soybean. Survival to the pupal stage was lower on kudzu than soybean overall, and the duration of the pupal stage was the same for both plants and foliage types. Percent survival to adulthood was highest for field soybean, followed by greenhouse soybean, greenhouse kudzu, and was lowest for field kudzu. One factor that potentially influenced larval survival and adult emergence was the amount of water sprayed into larval rearing dishes and pupation cups. Excessive amounts of water could have impeded movement of young larvae in Petri dishes and could have been fatal for pupating insects. Because the volume of water sprayed was not controlled during the experiment, the resulting variation could also account for differences in insect survival between studies conducted in 2004 and 2005. Survival to pupation in 2004 was 85.7% (field kudzu) and 42.9% (greenhouse soybean) (Chapter 1), compared to 46.7% (field kudzu) and 73.3% (greenhouse soybean) in 2005. Because similar leaf material was

collected in both years, differences in survival might be attributed to minor variations in rearing conditions and the transfer of younger, more teneral larvae to rearing chambers in 2005. Nevertheless, insect survival in 2005 was similar within a host plant, and differences in development times were recorded for kudzu only, where insects fed greenhouse foliage had faster development times than those fed field foliage.

Insect feeding preference did not differ for newly emerged adult beetles that were reared as larvae on one host plant and given a choice between field- and greenhouse-grown foliage of that plant. This was true for insects reared on kudzu and soybean, and reinforces the finding that differences in the measured physical and chemical leaf properties did not influence insect choice. However, it is possible that the leaf disks used in this experiment do not provide a reliable test of host specificity, as leaf disks are sometimes criticized for their failure to imitate natural host foliage (Kogan & Fischer 1991, Schmelz et al. 2001). In this case, any reliance on a search image for host plant recognition, which may be associated with the lobed shaped leaves (Renwick 1983) of kudzu, would not be available to insects presented with a leaf disk. On the other hand, it has been demonstrated that soybean leaf disks of 24 hours are not chemically different than recently produced disks (Kogan & Fischer 1991). Taken together, the equal consumption of field and greenhouse foliage likely demonstrates that plants grown in these conditions do not differ in an appreciable manner for *G. tredecimmaculata*.

In quarantine, low adult reproduction limited our ability to elucidate statistical differences in insect performance based on foliage quality. For example, during the first 26 days of the experiment (16 June to 11 July 2005), only 4.2 to 8.7% of paired adults per

treatment had reproduced, and average fecundity was both highly variable and non-significant (P = 0.7104). Minimum larval production for all treatments was 0, while maximums of 9 (field soybean), 28 (greenhouse kudzu), 86 (field kudzu), and 106 (greenhouse soybean) were recorded for the 26-day period. In an effort to increase the amount of reproduction, paired insects from different cages of the same treatment were combined. Cages of four individuals thus served as a reproductive unit during the next 23 days of the experiment (12 July to 4 August 2005). Average fecundity remained highly variable and non-significant (P = 0.6461), while percent reproduction per mating unit reached a maximum of 35.7% (field kudzu). One potential reason to account for low adult reproduction is the failure of sexually mature adults to find a suitable mate upon emergence. In other *Gonioctena* species, mating occurs shortly after emergence and reproduction occurs continuously throughout the year (Waloff & Richards 1958). If a critical period of mate finding is necessary, and was missed due to incorrect pairing of the sexes, this could prevent adults from successfully reproducing.

Adult oviposition preference was not easily determined for the same reasons as outlined above, specifically due to low adult reproduction. Since this experiment presented mating adults with field- and greenhouse-grown foliage of a host plant (kudzu or soybean), fecundity of adults following the experiment would be subject to variation based on exposure of reproducing females to two potential host plants at one time. Therefore, it was decided that oviposition preference would be tested at the end of the season, coincident with the termination of the fecundity and percent reproduction experiment. At this point, however, very few of the already limited number of adults

were reproducing. Nonetheless, results suggest that gravid females do not distinguish between field- and greenhouse-grown foliage when ovipositing or feeding, despite significant differences in leaf traits.

In a final set of experiments, adult beetles kept on one host plant for approximately 60 days were tested to evaluate the role that conditioning has on insect feeding preference (Peacock et al. 2003). It was hypothesized that insects previously fed on one host plant (kudzu or soybean) would preferentially feed on that plant when given a choice between the two, or eat more of the original host in a no-choice test (Peacock et al. 2003). In the first experiment, beetles given a choice between kudzu and soybean consumed both plants equally, showing no preference for one host over the other. In the second experiment, beetles presented with either kudzu or soybean consumed both foliage types and did not feed on one host plant more than the other. These results, contrary to our expectations, demonstrate that the ability of *G. tredecimmaculata* to feed on soybean is not altered by prior consumption of kudzu, while the same is true for feeding on soybean.

An important objective of this work was to understand the influence of plant growing conditions on the response and specificity of a potential biological control agent. The results demonstrate that statistical differences between foliar traits of field- and greenhouse-grown plants did not influence the feeding and reproduction of *G. tredecimmaculata* in quarantine conditions. This insect is able to cope with variations in leaf traits such as total carbon and nitrogen contents, toughness, water content, total phenolic content, and trichome density and length, with no appreciable cost to

development, survival, or reproduction. That the observed differences in leaf traits were not biologically significant in affecting the response of *G. tredecimmaculata* is an advantage for the insect, as natural variation in plant characteristics owing to climatic or latitudinal differences can occur with plants having a wide distribution (eg. trichome density, Levin 1973). Taken together, these results suggest that the use of greenhouse-grown foliage in host specificity testing may be acceptable, since it simulates the foliage that insects encounter in the field and elucidates a comparable response to field foliage.

The primary goal of this research was to further test the host specificity of a potential biological control agent for kudzu. Though not reported as a pest species in China (Talekar 1987), our work demonstrates that this insect is capable of completing its life cycle on soybean when evaluated in quarantine. In addition, choice tests showed that the insect did not distinguish between leaf disks of kudzu and soybean despite prior feeding on one of these hosts. Therefore, as an immediate consequence of this work, *Gonioctena tredecimmaculata* has been rejected as a control agent for kudzu in the United States.

Table 2.1. Trichome density and length for field and greenhouse foliage

Host Plant	Foliage Type	N	Number of Trichomes per mm^2 ($\bar{X}$ ± SEM)	Length of Trichomes ($\bar{X}$ mm ± SEM)[a]
G. max (soybean)	Field	12	3.5 ± 0.21 c	0.3 ± 0.02 ab
			*	*
	Greenhouse	11	1.5 ± 0.15 c	0.4 ± 0.02 a
P. lobata (kudzu)	Field	12	60.1 ± 4.31 a	0.4 ± 0.05 a
			*	*
	Greenhouse	12	26.3 ± 3.24 b	0.2 ± 0.01 b
F			97.76	8.21
df			3, 43	3, 43
P			< 0.0001	0.0002

In a column, means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)

*, t test indicates significant difference within host plant

[a] Data represents the average length of 10 trichomes per sample (N = 12)

Table 2.2. Toughness of leaves from the field and greenhouse

Host Plant	Foliage Type	N	Leaf Toughness ($\overline{X}$ ± SEM) [a]
Glycine max (soybean)	Field	30	56.0 ± 3.28 a
	Greenhouse	30	33.0 ± 1.28 b
Pueraria lobata (kudzu)	Field	30	57.6 ± 1.41 a
	Greenhouse	30	39.8 ± 1.95 b
F			32.25
df			3, 116
P			< 0.0001

[a] Leaf toughness is a measure of the force, in grams, needed to puncture a leaf. Data represent the average of two measurements per leaf. Means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)

Table 2.3. Water content of field and greenhouse foliage

Host Plant	Foliage Type	N	% Water ($\bar{X}$ ± SEM) [a]	
Glycine max (soybean)	Field	10	78.7 ± 0.74 b	*
	Greenhouse	10	80.9 ± 0.42 b	
Pueraria lobata (kudzu)	Field	10	79.9 ± 0.49 b	*
	Greenhouse	10	84.2 ± 0.51 a	
F			18.93	
df			3, 36	
P			< 0.0001	

* t test indicates significant difference within host plant ($P < 0.05$)

[a] Percent water is the difference between the wet and dry weight of three leaflets of a trifoliate. Analysis completed for arcsine transformed data; original mean percent and SEM are shown. Means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)

Table 2.4. Carbon and nitrogen content of leaves from the field and greenhouse

Host Plant	Foliage Type	N	% Carbon Content per 10 mg ($\overline{X}$ ± SEM)	% Nitrogen Content per 10 mg ($\overline{X}$ ± SEM)
Glycine max (soybean)	Field	10	48.7 ± 0.03 a	5.6 ± 0.01 b
	Greenhouse	10	48.2 ± 0.02 b	6.2 ± 0.01 a
Pueraria lobata (kudzu)	Field	10	48.7 ± 0.02 a	5.0 ± 0.03 c
	Greenhouse	10	44.1 ± 0.10 c	4.6 ± 0.02 d
F			1640.57	1420.45
df			3, 36	3, 36
P			< 0.0001	< 0.0001

In a column, means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range). Analysis completed for arcsine transformed data; original mean percent is shown

Table 2.5. Total phenolic content of field and greenhouse foliage

Host Plant	Foliage Type	N	Total Phenolic Content ($\overline{X}$ mg/ml ± SEM)
Glycine max (soybean)	Field	3	0.49 ± 0.050 a *
	Greenhouse	3	0.34 ± 0.007 a
Pueraria lobata (kudzu)	Field	3	0.39 ± 0.051 a
	Greenhouse	3	0.33 ± 0.009 a
F			4.11
df			3, 8
P			< 0.0487

Means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)

* t test indicates significant difference within host plant ($P < 0.05$)

Table 2.6. Fecundity of insects reared in 2004, overwintered in a 1.7 °C chamber, reanimated and kept on one host plant and foliage type starting 16 June 2005: first 26 days

Host Plant	Foliage Type	N [a]	Average Fecundity ($\overline{X}$ ± SEM) [b]	Range [c]	% Reproduction (N)
G. max (soybean)	Field	27	0.6 ± 0.41	0 – 9	7.4% (2)
	Greenhouse	23	4.7 ± 4.61	0 – 106	8.7% (1)
P. lobata (kudzu)	Field	24	3.6 ± 3.58	0 – 86	4.2% (2)
	Greenhouse	25	1.2 ± 1.12	0 – 28	8.0% (2)
F			0.46		
df			3, 95		
P			0.7104		

[a] Refers to the number of rearing containers with mating pairs of adults; most with 2 insects
[b] Fecundity data recorded from 16 June to 11 July 2005
[c] Minimum and maximum number of larvae produced during the 26 day period for each mating pair

Table 2.7. Fecundity of insects reared in 2004, overwintered in a 1.7 °C chamber, reanimated and kept on one host plant and foliage type starting 16 June 2005: next 23 days

Host Plant	Foliage Type	N [a]	Average Fecundity ($\overline{X}$ ± SEM) [b]	Range [c]	% Reproduction (N)
G. max (soybean)	Field	14	15.2 ± 8.70	0 – 96	28.6% (4)
	Greenhouse	14	15.1 ± 10.51	0 – 130	18.2% (2)
P. lobata (kudzu)	Field	11	14.9 ± 14.81	0 – 163	35.7% (5)
	Greenhouse	14	1.2 ± 0.58	0 – 6	21.4% (3)
F			0.56		
df			3, 49		
P			0.6461		

[a] Refers to the number of rearing containers with mating pairs of adults; most with 4 insects
[b] Fecundity data recorded from 12 July to 4 August 2005
[c] Minimum and maximum number of larvae produced during the 23 day period

Table 2.8. Development of insects reared on a single host plant and foliage type

Host Plant	Foliage Type	N	Larval Survival (N) [a]	Larval Period ($\bar{X}$ days ± SEM) [a]	Survival to Adulthood (N)	Time as Pupae ($\bar{X}$ days ± SEM) [b]	Adult Weight ($\bar{X}$ mg ± SEM)
G. max (soybean)	Field	15	66.7% (10)	6.5 ± 0.167 ab	53.33% (8)	8.4 ± 0.183 a	37.0 ± 2.063 a
	Greenhouse	15	73.3% (11)	6.9 ± 0.251 a	26.67% (4)	8.5 ± 0.646 a	32.6 ± 0.887 a
P. lobata (kudzu)	Field	15	46.7% (7)	6.9 ± 0.261 ab *	6.67% (1)	8.0 a	35.6 a
	Greenhouse	15	40.0% (6)	6.0 ± 0.000 b	20.00% (3)	9.3 ± 0.667 a	31.7 ± 2.875 a
F				3.07		1.00	1.24
df				3, 30		3, 12	3, 12
P				0.0429		0.4244	0.3398

In a column, means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)
*, t test indicates significant difference within host plant ($P < 0.05$). Data are for insects reared individually in Petri dishes.
[a] For survivors to pupation
[b] For adults that emerged from pupation

Table 2.9. Choice experiment with newly emerged adult insects reared as larvae on one host plant, and given a choice between field and greenhouse foliage of that host after emergence

Larval Host Plant	N	Field Area Consumed ($\overline{X}$ cm^2 ± SEM) [a]	Greenhouse Area Consumed ($\overline{X}$ cm^2 ± SEM)	T-Value [b]	Total Area Consumed ($\overline{X}$ cm^2 ± SEM)
G. max (soybean)	8	0.582 ± 0.267	0.922 ± 0.338	-1.31 (P = 0.356)	1.503 ± 0.523 a
P. lobata (kudzu)	8	1.443 ± 0.314	1.586 ± 0.289	-1.31 (P = 0.231)	3.029 ± 0.594 a
F					3.84
df					1, 14
P					0.0702

Means followed by a different letter are significantly different (P < 0.05; Tukey's Studentized Range)

[a] Total area given for 6 leaf disks = 4.298 cm^2; 2.149 cm^2 field, 2.149 cm^2 greenhouse; insects exposed for 21 hours

[b] T-Value of a Paired t Test computed using the difference between feeding values (field area eaten – greenhouse area eaten)

Table 2.10. No-choice feeding experiment with insects previously kept as adults on one host plant, then given either soybean or kudzu

Original Host Plant [a]	Test Plant [b]	N	Area Consumed ($\overline{X}$ cm^2 ± SEM) [c]
Glycine max (soybean)	soybean	10	0.576 ± 0.248 a
	kudzu	10	0.605 ± 0.214 a
Pueraria lobata (kudzu)	soybean	10	0.178 ± 0.060 a
	kudzu	10	0.799 ± 0.327 a
F			1.25
df			3, 36
P			0.3073

[a] Refers to the plant that insects were kept on as adults for approximately 60 days before the experiment

[b] Refers to the plant given during the experiment

[c] Total area given for 6 leaf disks = 5.170 cm^2; insects exposed to foliage for 17.5 hours. Means followed by a different letter are significantly different ($P < 0.05$; Tukey's Studentized Range)

Table 2.11. Adult choice experiment with insects previously kept as adults on one host plant, then presented with 3 soybean leaf disks and 3 kudzu leaf disks

Original Host Plant [a]	N [b]	Soybean Area Consumed ($\overline{X}$ cm^2 ± SEM) [c]	Kudzu Area Consumed ($\overline{X}$ cm^2 ± SEM)	T-Value [d]
Glycine max (soybean)	7	0.92 ± 0.162	1.24 ± 0.166	2.29 (P = 0.0617)
Pueraria lobata (kudzu)	6	0.78 ± 0.251	1.45 ± 0.128	2.34 (P = 0.0668)

[a] Refers to the plant that insects were kept on as adults for approximately 60 days before the experiment

[b] Represents number of insects out of 8 in each treatment that fed on leaf disks

[c] Total area given for 6 leaf disks = 5.102 cm^2; 2.551 cm^2 soybean, 2.551 cm^2 kudzu; insects exposed for 18.5 hours

[d] T-Value of a Paired t Test computed using the difference between feeding values (kudzu area eaten – soybean area eaten)

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