

## Biology and Preliminary Host Range Assessment of Two Potential Kudzu Biological Control Agents

MATTHEW J. FRYE,<sup>1</sup> JUDITH HOUGH-GOLDSTEIN,<sup>1,2</sup> AND JIANG-HUA SUN<sup>3</sup>

Environ. Entomol. 36(6): 1430-1440 (2007)

**ABSTRACT** Two insect species from China, *Gonioctena tredecimmaculata* (Jacoby) (Coleoptera: Chrysomelidae) and *Ornataleides (Mesalcidodes) trifidus* (Pascoe) (Coleoptera: Curculionidae), were studied in quarantine in the United States as potential biological control agents for kudzu, *Pueraria montana* variety *lobata* (Willd.) Maesen and S. Almeida. Adults of *G. tredecimmaculata* were ovoviparous and reproduced throughout the summer, producing offspring that had an obligate adult diapause. In no-choice tests, adult and larval *G. tredecimmaculata* rejected most of the plant species tested, but consumed foliage and completed their life cycle on soybean (*Glycine max* L. Merr.) and on a native woodland plant, hog-peanut (*Amphicarpaea bracteata* L. Fernald), which are in the same subtribe as kudzu (Glycininae). Insects showed similar responses to field- and greenhouse-grown soybean and kudzu foliage, despite measurable differences in leaf traits: field-grown foliage of both plants had greater leaf toughness, higher total carbon content, higher trichome density, and lower water content than greenhouse foliage. *O. trifidus* adults also rejected most of the plants tested but fed on and severely damaged potted soybean and hog-peanut plants in addition to kudzu. Further tests in China are needed to determine whether these species will accept nontarget host plants under open-field conditions.

**KEY WORDS** weeds, invasive species, host plant specificity, classical biological control, risk analysis

Kudzu, *Pueraria montana* variety *lobata* (Willd.) Maesen and S. Almeida, was first introduced from Asia 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). During the 1930s and 1940s, kudzu was widely distributed in the southeastern United States by the Soil Conservation Service for erosion control (Tabor and Susott 1941, Mitich 2000). By the 1950s, however, it was recognized that kudzu can spread rapidly, climbing over existing foliage to kill trees and saplings through light competition (Munger 2002). Since its introduction into the United States, kudzu has been responsible for substantial annual damage to lumber and pulpwood industries and costly maintenance of rights of way and power lines, in addition to likely impacts on native plants (Shurtleff and Aoyagi 1985, Forseth and Innis 2004). A current and pressing issue is the recent introduction of *Phakopsora pachyrhizi* Sydow (Uredinales: Pucciniaceae), the causal agent of soybean rust, into the United States. The ability of this pathogen to overwinter on kudzu poses a potential threat to agri-

culture (Perez-Hernandez et al. 1994, Pivonia and Yang 2004).

Complete defoliation over several growing seasons depletes kudzu root reserves, which can kill the plant (Brender 1961). Thus, overgrazing by cattle or goats and persistent weeding or mowing have been proposed as possible techniques to eliminate kudzu (Bonsi et al. 1991). Such 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 applied for multiple years or combined with other control methods (Harrington et al. 2003). The lack of mechanical and chemical strategies for large-scale control of kudzu make it a possible target for classical biological control (Britton et al. 2002).

In China, >100 phytophagous insect species in six feeding guilds were found on kudzu (Sun et al. 2006). Of these, several were recommended for further host-range testing based on preliminary tests in China, including the species reported on here, *Gonioctena tredecimmaculata* (Jacoby) (Coleoptera: Chrysomelidae) and *Ornataleides (Mesalcidodes) trifidus* (Pascoe) (Coleoptera: Curculionidae).

In a recent taxonomic review, Bezděk (2002) concluded that the species usually identified as *G. tredecimmaculata* is actually comprised of five different species. Based on distribution and morphology, however, the species studied here is the true *G. tredecim-*

<sup>1</sup> Department of Entomology and Wildlife Ecology, University of Delaware, Newark, DE 19716-2160.

<sup>2</sup> Corresponding author: Department of Entomology and Wildlife Ecology, 531 S. College Ave., Newark, DE 19716-2160 (e-mail: jhough@udel.edu).

<sup>3</sup> State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China.

*mmaculata*. The native range of this species includes China and Taiwan (Bezděk 2002). Zheng et al. (2005) listed *G. tredecimmaculata* as oligophagous, based on several Chinese references, but did not indicate which hosts might be accepted other than kudzu. This chrysomelid is a kudzu defoliator, feeding on leaf tissue as larvae and as adults. Adults are oval, ≈6–8 mm long, with an ornate pattern of black spots on the glabrous, lustrous, orange to reddish-brown thorax and elytra (Jacoby 1888, Bezděk 2002).

*Ornatalcides trifidus* is in the weevil tribe Mecesolobini, which is in need of comprehensive revision (Witt et al. 2004), and therefore the generic placement of the species may change. We follow the nomenclature of Alonso-Zarazaga and Lyal (1999) in referring to this species as *O. trifidus*, while recognizing that other authors follow Pajni and Dhir (1987), who proposed establishing the genus *Mesalcidodes* Voss 1958 with *Mesalcidodes trifidus* (Pascoe) as the type species. Earlier literature generally used the generic name *Alcidodes*. *O. trifidus* has been reported in China, Japan, and Taiwan. Tayutivutikul and Kusigemati (1992a) reported that *O. trifidus* was monophagous on kudzu based on their observations in Japan, but cited several Japanese sources listing *Pyrus* and *Citrus* spp. as possible hosts. In addition, Zheng et al. (2004) listed *Lespedeza* spp. as possible hosts for *O. trifidus*. The relatively large (≈8–10 mm long), boldly patterned black and white adults of this weevil are sometimes called “panda weevils” in Japan. They reportedly lay their eggs into spiral notches cut in the base of kudzu stems (J.H.S., unpublished data). Larvae feed internally, causing the production of large stem galls (Tayutivutikul and Kusigemati 1992b).

Kudzu is in the family Fabaceae (=Leguminosae), subfamily Faboideae, tribe Phaseoleae (USDA–GRIN 2006). The subtribe Glycininae is sometimes considered polyphyletic because of similarities between its members and plants in other Phaseoleae subtribes (Viviani et al. 1991, Bruneau et al. 1994, Kajita et al. 2001). Other plants in the Glycininae present in North America are soybean, *Glycine max* L. Merr., which was introduced into the United States as a crop plant from Asia in the early part of the 20th century (Turnipseed and Kogan 1976); hog-peanut, *Amphicarpea bracteata* L. Fernald, an herbaceous woodland annual, native to North America; and four species of *Cologania* native to the southwestern United States.

Here we report the results of biological studies on *G. tredecimmaculata* and *O. trifidus* in quarantine. Host-range studies focused on plants within the subfamily Faboideae, especially soybean and hog-peanut. Additional trials compared *G. tredecimmaculata* consumption of greenhouse-grown soybean and kudzu foliage to field-grown foliage to test the hypothesis that field plants might differ from greenhouse plants because of exposure to environmental stresses and attack by herbivores, predators, and pathogens in the field (Woodhead 1981, Cipollini 1997, Underwood et al. 2002). Results of measurements of various leaf traits of greenhouse- and field-grown kudzu and soybean are also reported.

## Materials and Methods

**Test Plants.** Kudzu plants from recently rooted nodes were collected in Dover and Glasgow, DE, in May and June 2004 and transplanted into plastic pots (30.5 cm diameter) with Pro-mix (Premier Horticulture, Quebec, Canada). Subsequent plants were grown in the greenhouse by burying shoot nodes produced by these plants in new pots until they rooted (Shurtleff and Aoyagi 1985). Additional foliage was collected from a kudzu patch in Fair Hill, MD.

For host-range tests with *G. tredecimmaculata* in 2004, seeds of bush bean and pole bean (two varieties of *Phaseolus vulgaris* L.; Blue Lake 274 and Kentucky Wonder Brown, respectively), soybean (CL48RR), and cowpea (*Vigna unguiculata* L. Walp., California blackeye) were purchased locally, and seeds of sensitive joint-vetch (*Aeschynomene virginica* L. Britton et al., AY104A) were purchased from Seedland.com (Wellsborn, FL). Plantings were made once per week, and plants were grown in 15-cm-diameter plastic pots in Pro-mix. Hog-peanut foliage was collected from the field at White Clay Creek State Park, Newark, DE, with trifoliolate leaves transported to the laboratory in plastic bags.

Host-range tests with adult *O. trifidus* were conducted in 2005 and used greenhouse-grown plants from the same seeds as above for bush bean, cowpea, and soybean. Hog-peanut plants were grown from seeds collected in 2004 from the White Clay Creek site. In addition, seeds of jack-bean (*Canavalia ensiformis* L. DC.), pigeonpea (*Cajanus cajan* L. Millsp.), *Erythrina abyssinica* Lam. ex DC., and hyacinth-bean (*Lablab purpureus* L. Sweet) were purchased from The Banana Tree (Easton, PA). Pea (*Pisum sativum* L., Burpeana Early) and alfalfa (*Medicago sativa* L., 53H81) seeds were purchased locally. Seeds were planted in 15-cm-diameter plastic pots in the greenhouse, watered daily, and fertilized at recommended rates every 2 wk.

For trials comparing greenhouse- and field-grown foliage, kudzu seedpods were collected in February 2005 from the field site in Fair Hill, MD. Pods were hulled, and seeds were stored at room temperature. Kudzu was planted in the greenhouse twice: on 5 and 20 May 2005. For both plantings, seeds were treated to break seed-coat dormancy using 10-s immersion in boiling water (Susko et al. 2001) and planted the next day. Field kudzu foliage was taken from the field site where seeds had been collected. Soybean seeds (RT-3940 N) were purchased locally and used for plantings in both the greenhouse and field. In the greenhouse, soybeans were planted every 7 d from May to August and kept in the same room as the kudzu plants, under natural light. Seeds were sown in plastic pots (9.5 by 9.5 by 8.5 cm) with Pro-mix, watered twice daily, and fertilized every 2 wk. In the field, soybean seeds were planted in rows 76 cm apart at six plants per 30.5 cm, with 150,000 seeds/0.4 ha, on 4 and 13 May 2005. Both plantings were treated with glyphosate on 25 May 2005.

Similar criteria were used to select foliage for all of the field versus greenhouse experiments, including leaf trait measurements and insect trials. Tender young leaves (preferred by *G. tredecimmaculata*) were cut off at the base of the petiole with pruning shears and placed in a plastic bag. For soybean, the two unfolded trifoliolate leaves at the apical end of a V2 (vegetative growth stage with three nodes and two unfolded leaflets; McWilliams et al. 1999) plant were collected from field and greenhouse plants. Later in the season when all field plants were older than the V2 stage, the two most apical unfolded leaves were collected, but never from reproductive plants. For kudzu, light-green newly expanded leaves near the end of an elongating shoot were collected from the field. Leaflets were  $\approx 8$ –10 cm long. Newly expanded leaves on a shoot were also collected from greenhouse kudzu. After two leaves had been removed, all soybean plants and greenhouse-grown kudzu plants were cut at the base and discarded to prevent the use of plants in later experiments that may have been chemically induced. Leaves were transported to the quarantine facility or to the laboratory for leaf trait measurements in a cooler with an ice pack.

**Biology and Preliminary Host Range Assessment of *G. tredecimmaculata*.** Approximately 65 adults of *G. tredecimmaculata* were collected on kudzu in Xuancheng, Anhui Province, China, and sent to the USDA-ARS Beneficial Insects Introduction Research Facility, Newark, DE, in June 2004. Forty-eight insects arrived alive and were kept in a quarantine room at  $25.6 \pm 0.02^\circ\text{C}$ , 16 L: 8 D in screen cages (47 by 41 by 35 cm) with potted kudzu plants or kudzu foliage collected from the field. Neonate larvae (from eggs that had been laid and hatched overnight) were collected each day from adult cages using a fine-tip paintbrush. Larvae were transferred to large plastic tubs (32 by 26 by 10 cm) containing a layer ( $\approx 5$  cm deep) of moist Pro-mix and kudzu foliage. Pupation occurred in the Pro-mix, and newly emerged adults were collected daily.

Thirty neonate larvae were placed in groups of three in 10 by 1.5-cm petri dishes to determine the development time and number of larval instars. Larvae were provided with excess kudzu foliage, and dishes were searched daily for head capsules. Once full-grown larvae began wandering, they were transferred to 478-ml cardboard containers with moist Pro-mix for pupation. Containers were monitored daily for adult emergence.

To determine adult kudzu consumption and time to diapause (defined as when adult insects stopped feeding and dug into the Pro-mix), single pairs of newly emerged (F1) beetles were placed in small plastic tubs (8 by 12.5 by 7 cm,  $N = 6$ ) with a 2.5-cm layer of Pro-mix and provided with kudzu foliage. Leaves were collected from each tub periodically, and individual leaflets were press-dried, photocopied, and scanned into the computer as black and white images (HP Scanjet 3970; Hewlett-Packard, Palo Alto, CA). ImageJ software (ImageJ 1.36 for Windows; National Institutes of Health, Bethesda, MD) was used to de-

termine leaf area eaten, as the difference between the original leaf area (recreated with a black permanent marker) and leaf area remaining after insect consumption. In an additional trial, newly emerged adult beetles were placed individually in 10 by 1.5-cm petri dishes lined with moist filter paper ( $N = 7$ ), and adults were given a single kudzu leaflet every day for 7 d. Amount of leaf area consumed was determined by the method described above.

For adult no-choice tests, newly emerged (F1) beetles were placed in groups of six adults each on a host plant, which was enclosed in a 33-cm-tall by 15-cm-diameter cylindrical cage of 0.12-cm gauge plastic film topped with nylon netting. Potted plants were used for pole bean, bush bean, joint-vetch, cowpea, and soybean, whereas fresh cut trifoliolate leaves of kudzu and hog-peanut in aqua picks were placed in pots with Pro-mix. Host plants or foliage were changed every 4 d. Controls (to determine insect ability to survive in the absence of any host plant) consisted of pots containing only moist Pro-mix and a dental wick saturated with water. Two groups of insects in separate cages (12 beetles total) were used for most host plants and for the control; four groups (24 beetles total) were used for soybean; and one group (six insects) for hog-peanut. Survival to diapause, survival over the winter (to 2005), and the amount of foliage eaten (determined as above) were recorded. Where all available foliage was consumed, the amount eaten was estimated.

For larval no-choice tests, neonate larvae collected from kudzu foliage in adult rearing cages were placed individually in petri dishes lined with moistened filter paper. Larvae ( $N = 6$ –14 per host plant) were provided with leaflets of pole bean, bush bean, cowpea, kudzu, soybean, hog-peanut, or moistened filter paper only as a control. New leaflets were added every other day, and old leaflets were removed and press-dried to determine larval leaf area consumption using the method described above. Dishes were searched daily for head capsules. Full-grown (wandering) larvae were placed in 163-ml plastic cups containing moist Pro-mix for pupation.

A larval choice test to compare consumption of kudzu and soybean was conducted with neonate larvae placed individually in petri dishes lined with moist filter paper ( $N = 8$ ). Insects were presented with similar-sized, overlapping leaflets of both kudzu and soybean during the larval period. Leaflets were replaced every other day, and leaf area consumption was measured for each host plant using the method described above.

By July 2004, it was apparent that F1 beetles do not reproduce in their first year. Because we wished to determine whether adult beetles would lay eggs on soybean in addition to kudzu, all remaining parental beetles from China were divided at random into two cages on 19 July 2004. Twelve beetles were placed in a cage with kudzu foliage only, and the other 12 beetles were put in a cage containing soybean plants only. Beetles were kept separate and larvae were collected

daily from each cage for 1 wk, after which all insects were returned to the cage with kudzu.

A step-down process was initiated in October 2004 to overwinter all remaining parental and F1 beetles. Insects were put in self-sealing plastic bags containing moistened sphagnum moss, placed in a dark chamber at 13°C for 14 d, and moved to another dark chamber at 1.7°C for the winter (protocol suggested by D. Palmer, Phillip Alampi Beneficial Insect Rearing Lab, New Jersey Department of Agriculture, Trenton, NJ). In June 2005, the procedure was reversed and beetles were returned to the 25°C rearing room where they were removed from the plastic bags and given fresh foliage.

***Gonioctena tredecimmaculata* Response to Field Versus Greenhouse Foliage.** In 2005, eggs or neonate larvae (before feeding) produced by overwintered F1 beetles were transferred individually with a fine-tipped paintbrush to a petri dish (10 by 1.5 cm) lined with moist filter paper and given either soybean or kudzu foliage from plants grown in either the greenhouse or the field ( $N = 15$  per host and foliage type, total of 60 dishes). Dishes were checked daily for head capsules, and full-grown larvae were placed in 163-ml plastic cups containing Pro-mix for pupation. Adult beetles were weighed on emergence.

To determine whether adult beetles with no prior feeding experience preferred field or greenhouse foliage, newly emerged adults from larvae fed on soybean or kudzu in the above experiment were placed in the center of a petri dish and given a choice of three field and three greenhouse leaf disks (1.1 cm diameter) of the same plant species (soybean or kudzu) that they had been fed as larvae ( $N = 8$  for each plant species). Leaf disks were arranged alternately and evenly spaced on moistened filter paper around the edge of the dish. Insects were left overnight (21 h), and foliage consumption was determined as above. In this test, the leaf area remaining after insect feeding was compared with a set of six leaf disks kept under the same conditions but not exposed to insects. For example, the area of three field-grown soybean leaf disks after insect feeding was subtracted from the area of three undamaged field-grown soybean leaf disks set up at the same time, and the difference was recorded as leaf area consumed.

To determine the beetles' reproductive output on different foliage types, overwintered (F1) adults were placed in male-female pairs in plastic tubs containing a 2- to 3-cm layer of Pro-mix and two trifoliolate leaves. Size was used to separate the smaller males from females, although the species exhibits no distinct sexual dimorphism (Bezdek 2002, Frye 2006), and some may have been incorrectly assigned. Between 23 and 27 pairs were given foliage from each plant species (kudzu or soybean) and growing condition (field or greenhouse) for a total of 109 pairs. Insects were checked daily from 16 June to 11 July 2005. Eggs and larvae produced each day were removed, and foliage was changed or added every other day.

Paired insects that had been given the same foliage type (e.g., field-grown kudzu) were combined into

groups of four insects on 12 July 2005. On 10 August, 10 of these groups from each foliage type were given one field and one greenhouse leaf of their previous host (kudzu or soybean), overlapping in the middle, to test oviposition preference. Insects were left to feed and reproduce for 24 h, and consumption and oviposition were assessed for each leaf.

A number of the paired and grouped insects in the above tests did not produce any offspring, and on 25 August 2005, beetles that had still not reproduced were combined into two containers. Insects from field and greenhouse soybean were combined and given greenhouse soybean, and beetles from field and greenhouse kudzu were given field kudzu. On 30 August, all of these beetles (which still had not reproduced) were placed in ethyl alcohol for later dissection. A total of 111 beetles were dissected to determine the sex ratio and the reproductive state of females. Females with developing larvae present in their abdomens were recorded as gravid.

**Foliar Traits of Field Versus Greenhouse Plants.** To determine carbon and nitrogen content,  $\approx 30$  trifoliolate leaves of each foliage type (field- and greenhouse-grown kudzu and soybean) were collected. Leaflets were cut at the petiole, and all leaflets of a single foliage type were pooled, dried for 6 h at 64°C, and ground into a fine powder using a mortar and pestle. Ten samples of 10 mg each were processed with an Elementar Vario-Max CN analyzer (Elementar America, Mt. Laurel, NJ). Samples were analyzed by combustion using the Dumas Technique, and results are reported as percent composition of each element by weight.

To determine leaf phenolic content,  $\approx 30$  trifoliolate leaves of each foliage type were prepared as powdered samples, as above. Dried leaf material (0.25 g) was homogenized in 10 ml of 99.9% methanol in a 50-ml test tube using a Ryobi 3/8-in Variable Speed Drill D40 (Techtronic Industries Co., Tsuen Wan, New Territories, China) and a Teflon tipped pestle for  $\approx 3$  min with the test tube immersed in a water/ice bath. Extracted samples were centrifuged for 10 min at 4,000 rpm, and the supernatant was transferred to a 25-ml volumetric flask and brought up to mark with methanol. Once prepared, 500  $\mu$ l of the sample was combined with 3,050  $\mu$ l distilled H<sub>2</sub>O and 150  $\mu$ l Folin-Denis reagent and vortexed. Next, 300  $\mu$ l of 35% Na<sub>2</sub>CO<sub>3</sub> was added, and the sample was vortexed. Absorbance was noted after 15 min at 730 nm in a diode array spectrophotometer (Agilent 8453; Agilent Technologies, Palo Alto, CA). The concentration of phenolics was determined by comparing sample absorbance to the absorbance of tannic acid standards of known concentrations diluted with methanol (0.2, 0.4, 0.6, and 0.8 mg/ml) using UV-Visible ChemStation Software (Agilent Technologies). The blank for spectrophotometric analysis contained all reagents excluding sample extract.

Ten trifoliolate leaves of each foliage type were collected to determine leaf water content. Leaflets were removed, and the three leaflets were weighed together to determine fresh weight, dried for 6 h at

64°C, and weighed again. Percent water content was  $(1 - [\text{dry weight}/\text{fresh wt}]) \times 100$ .

Leaf toughness and trichome density and length were measured halfway from the mid-vein to the leaflet edge and halfway from the top to the bottom of a leaflet. Leaf toughness, or the amount of force (in grams) necessary to puncture a hole in the leaf, was measured with a TA.XT2i Texture Analyzer (Texture Technologies, Scarsdale, NY). Leaflets were removed from 15 leaves, and two measurements were taken and averaged per leaflet. A total of 30 leaflets were analyzed per foliage type (2 leaflets per leaf) with a 2.0-mm flat tip probe (as in Kudo 2003) and a test speed of 1.5 mm/s until the sample ruptured.

To determine trichome density, six trifoliolate leaves of each foliage type were collected, leaflets were removed, and digital images of the adaxial (upper) surface were taken (Leica DC 100; Leica Microsystems, Bannockburn, IL) at low and high magnification (field size of 180 and 7 mm<sup>2</sup>, respectively) for 12 leaflets. Images were printed and trichomes were counted. To correct for differences in field size, trichome densities were divided by the magnification to yield trichomes per square millimeter.

Ten trichomes per image (120 per foliage type) were randomly selected, and their lengths were measured. The ratio between the field size of the hard copy image and the field size of the image on the computer screen was used to determine the actual length of the trichome, which was averaged for each leaflet.

***Ornaticides trifidus* Adult No-choice Tests.** Adult *O. trifidus* were collected on kudzu in Xuancheng, Anhui Province, China, and sent to the Newark, DE, quarantine facility in June 2005. The 107 weevils that survived shipping were placed in a large screen cage (47 by 41 by 35 cm) with potted greenhouse-grown kudzu plants or bouquets of field-collected kudzu terminals ( $\approx 50$  cm long) placed in 250-ml Erlenmeyer flasks filled with water and plugged with cotton. Kudzu terminals were used because adults in China were found feeding, mating, and ovipositing primarily on tender new shoots of the plant.

Because insects did not successfully reproduce in quarantine, no-choice tests were conducted between June and August 2005 using the adult insects sent from China. For each test, 10 weevils were selected at random from the large cage and placed into a smaller screen cage (33 by 41 by 46 cm) with a single potted test plant. Three cages were used for tests with soybean, whereas two cages were used for tests with all other plant species. One or two of the potential host plants were tested at a time, and at least two cages with kudzu terminals or potted plants were included as a control and assessed at the same time as test plants. Weevils were kept with a single plant species for 5 d, after which feeding damage was assessed qualitatively, and surviving adults were counted and placed back in the large cage with kudzu terminals. After 2 d, a new trial was begun. In the soybean no-choice trial, the number of weevils found on soybean was recorded after 1 d and compared with weevil numbers on kudzu

foliage. A choice test was also conducted in which 10 weevils were placed in a cage containing both a soybean plant and a bouquet of kudzu terminals ( $N = 3$ ). After 3 d, an additional kudzu bouquet was added to each cage. Plant damage to each host was assessed after 2 and 5 d.

**Data Analysis and Voucher Specimens.** Data from the *G. tredecimmaculata* larval no-choice test were analyzed using analysis of variance (ANOVA) followed by Tukey's studentized range (honestly significant difference [HSD]) test. Paired *t*-tests were used to compare foliage consumption in the *G. tredecimmaculata* larval and adult choice tests, and *t*-tests were used for adult no-choice tests. Total carbon and nitrogen contents, total phenolic content, water content, leaf toughness, trichome density and length, fecundity, larval development, duration of the pupal stage, and adult weight data were analyzed with ANOVAs followed by Tukey's HSD test to evaluate differences between plant species and growing conditions; *t*-tests were also used for comparisons between growing conditions within a plant species. Data from percent water and total nitrogen content determinations were arcsine transformed, because percentages fell outside the range of 30–70% (Snedecor and Cochran 1967). Paired *t*-tests were used to analyze *G. tredecimmaculata* feeding in the adult choice test comparing field to greenhouse foliage. The SAS System (Version 9.1; SAS Institute, Cary, NC) was used for all statistical procedures. Voucher specimens of *G. tredecimmaculata* and *O. trifidus* have been deposited in the Insect Reference Collection of the Department of Entomology & Wildlife Ecology, University of Delaware, Newark, DE.

## Results

**Biology and Preliminary Host Range Assessment of *G. tredecimmaculata*.** Beetles from China kept under quarantine conditions in 2004 showed a fairly high reproductive rate, with the 48 adults that survived the shipping process (reduced to 26 adults by 10 July) producing >700 offspring between June and September. No definitive external morphological differences between the sexes were found (Frye 2006), making it difficult to determine individual fecundity. However, individual females were observed to produce between two and six larvae per day for extended periods during the summer.

*Gonioctena tredecimmaculata* females were ovoviparous (defined as embryonic development that is completed, at least in part, before larval deposition), with eggs hatching within  $\approx 10$  min of deposition on leaf surfaces. Eggs were 2–3 mm long and consisted of fully developed larvae surrounded by a thin membrane. Larvae grew rapidly, passing through four instars in a total of  $5.6 \pm 0.1$  d, and the pupal stage lasted  $9.6 \pm 0.1$  d ( $N = 30$ ). Of the larvae reared on kudzu foliage, 71% emerged as adults (273 of 383). Adults from this generation did not mate or reproduce, but fed for an average of  $15.0 \pm 0.4$  d ( $N = 6$ ) and then dug into the soil and remained there until the fall, when

Table 1. Survival and leaf area consumed by *G. tredecimmaculata* adults in a no-choice host specificity test

Host plant	N <sup>a</sup>	Survival to diapause (%)	Survival to 2005 (%)	Amount consumed per insect (mean cm <sup>2</sup> )
<i>Pueraria montana</i> var. <i>lobata</i> (kudzu)	12	91.7	91.7	~75 <sup>b</sup>
<i>Glycine max</i> (soybean)	24	75.0	37.5	~70 <sup>b</sup>
<i>Amphicarpaea bracteata</i> (hog-peanut)	6	50.0	50.0	~70 <sup>b</sup>
<i>Phaseolus vulgaris</i> (pole bean)	12	16.7	0	1.1
<i>Phaseolus vulgaris</i> (bush bean)	12	0	0	4.7
<i>Aeschynomene virginica</i> (joint-vetch)	12	0	0	0
<i>Vigna unguiculata</i> (cowpea)	12	0	0	0
Water only	12	0	0	NA <sup>c</sup>

<sup>a</sup> Total number of insects (kept in groups of six); foliage changed every 4 d.

<sup>b</sup> Estimated (entire leaves consumed).

NA, not applicable.

they were placed in cold storage. Of these adults, 85% survived to 2005. Thirty-eight percent of the beetles shipped to the quarantine facility in June 2004 survived until the fall (18 of 48) and were placed in cold storage, but none of these individuals survived to 2005.

Pairs of beetles in 2004 consumed  $10.5 \pm 0.5$  cm<sup>2</sup> ( $N = 6$ ) of kudzu foliage per day, or  $\approx 5.2$  cm<sup>2</sup> per insect per day. Single beetles given kudzu for 7 d consumed an average of  $7.9 \pm 0.4$  cm<sup>2</sup> ( $N = 7$ ) of foliage per day.

In no-choice tests using newly emerged (F1) adults in 2004, survival was greatest on kudzu, but more than one third of the beetles given only soybean or hog-peanut foliage survived to diapause and to 2005 (Table 1). No beetles survived to diapause on bush bean, joint-vetch, cowpea, or water alone, and one individual given pole bean foliage survived to diapause but not to 2005. A total of  $<5$  cm<sup>2</sup> of foliage was consumed per insect for all plants except kudzu, soybean, and hog-peanut (Table 1).

Neonate larvae placed on pole bean, bush bean, and cowpea foliage survived for only  $\approx 2$  d, as did insects given water only. Survival to adulthood was greatest for larvae given kudzu, but  $>20\%$  survived to adulthood on soybean and 50% on hog-peanut foliage (Table 2). Larval development time was longer on soybean than on kudzu or hog-peanut, but the pupal period on soybean was shorter than on kudzu (Table 2). Total leaf area consumed by larvae that survived to pupation on single hosts did not differ for kudzu

( $16.3 \pm 0.6$  cm<sup>2</sup>) and soybean ( $14.8 \pm 1.0$  cm<sup>2</sup>;  $t = 1.66$ ,  $P = 0.2160$ ,  $N = 8$  per host). When given a choice of kudzu or soybean foliage, larvae consumed some of each, but ate significantly more kudzu ( $14.0 \pm 1.5$  cm<sup>2</sup>) than soybean ( $2.5 \pm 1.5$  cm<sup>2</sup>) foliage ( $t = 3.95$ ,  $P = 0.0055$ ;  $N = 8$ ).

When adult beetles in the parental generation from China were divided into two groups after  $\approx 1$  mo on kudzu in quarantine, the 12 beetles that were placed on soybean foliage all burrowed into the soil, whereas the 12 on kudzu continued to reproduce, producing a total of 69 larvae (9.9/d) during the next 7 d.

***Goniocentrus tredecimmaculata* Response to Field Versus Greenhouse Foliage.** On average, larval development took an additional day on soybean compared with kudzu greenhouse-grown foliage (Table 3). Development was slightly more rapid for insects reared on greenhouse compared with field kudzu foliage ( $t = 3.02$ ,  $P = 0.0116$ ). Survival to adulthood was higher for insects reared on soybean than kudzu, and no differences were found for the duration of the pupal stage or adult weights at emergence (Table 3). When given a choice between the two, newly emerged adult insects showed no preference for field or greenhouse leaf disks of kudzu ( $t = -1.31$ ,  $P = 0.231$ ,  $N = 8$ ) or of soybean ( $t = -1.31$ ,  $P = 0.356$ ,  $N = 8$ ).

For each plant species and growing condition,  $<9.0\%$  of the overwintered F1 adults that were kept in pairs from 16 June to 11 July produced offspring. Of the 23–27 pairs kept on each of the different foliage

Table 2. Survival and development (means  $\pm$  SEM) of *G. tredecimmaculata* larvae in a no-choice host specificity test

Host plant	N	Survival to pupation (%)	Larval period <sup>a</sup> (d)	Survival to adult (%)	Pupal period <sup>b</sup> (d)
<i>P. montana</i> (kudzu)	14	85.7	5.8 $\pm$ 0.1c	64.3	10.2 $\pm$ 0.2a
<i>G. max</i> (soybean)	14	42.9	7.5 $\pm$ 0.3a	21.4	9.3 $\pm$ 0.3b
<i>A. bracteata</i> (hog-peanut)	8	50.0	6.0 $\pm$ 0.0b	50.0	10.0 $\pm$ 0.0ab
<i>P. vulgaris</i> (pole bean)	7	0	—	—	—
<i>P. vulgaris</i> (bush bean)	6	0	—	—	—
<i>V. unguiculata</i> (cowpea)	6	0	—	—	—
Water only	8	0	—	—	—
F			21.34		5.20
df			2.19		2.13
P			$<0.0001$		0.0219

Means within a column followed by the same letter are not significantly different ( $P < 0.05$ ; ANOVA, Tukey's studentized range test).

<sup>a</sup> For survivors to pupation.

<sup>b</sup> For survivors to adulthood.

**Table 3.** Survival and development (means  $\pm$  SEM) of *G. tredecimmaculata* reared on kudzu or soybean grown in the field or greenhouse

Plant species	Growing conditions	N	Percent survival (egg to pupa)	Larval period (d) <sup>a</sup>	Percent survival (egg to adult)	Pupal period (d) <sup>b</sup>	Adult weight (mg)
Kudzu	Field	15	46.7	6.9 $\pm$ 0.3ab	6.7	8.0a	35.6a
	Greenhouse	15	40.0	6.0 $\pm$ 0.0b*	20.0	9.3 $\pm$ 0.7a	31.7 $\pm$ 2.9a
Soybean	Field	15	66.7	6.5 $\pm$ 0.2ab	53.3	8.4 $\pm$ 0.2a	37.0 $\pm$ 2.1a
	Greenhouse	15	73.3	6.9 $\pm$ 0.3a	26.7	8.5 $\pm$ 0.6a	32.6 $\pm$ 0.9a
F				3.07		1.00	1.24
df				3,30		3,12	3,12
P				0.0429		0.4244	0.3398

Means within a column followed by the same letter are not significantly different ( $P < 0.05$ ; ANOVA, Tukey's studentized range).

<sup>a</sup> For survivors to pupation.

<sup>b</sup> For survivors to adulthood.

\* *t*-Test indicates significant difference within host plant ( $P < 0.05$ ).

types, only 1 or 2 pairs per foliage type reproduced (Table 4). There were no significant differences in the reproductive output of adult insects on the different plant species (kudzu and soybean) or growing conditions (field and greenhouse).

Of the 10 groups of four insects that were used per foliage type (total of 40) to compare oviposition on field versus greenhouse foliage, only 7 groups reproduced during the 24-h test period. Of the insects that did reproduce, beetles from field-grown soybean ( $N = 3$ ) fed and deposited offspring on both field (5 larvae) and greenhouse (11 larvae) foliage. Beetles that reproduced from greenhouse-grown soybean ( $N = 2$ ) fed only on greenhouse foliage, but deposited offspring on both field (four larvae) and greenhouse (five larvae) foliage. Beetles from field-grown kudzu ( $N = 1$ ) fed only on field foliage, but deposited offspring on both field (two larvae) and greenhouse (one larva) foliage. None of the beetles from greenhouse kudzu reproduced, but adults fed on both foliage types.

Of the beetles dissected in the fall after failing to reproduce all summer, 27 of the adults kept on soybean were male and 22 were female. Nine of the 22 females (40.9%) were gravid, i.e., had developing larvae present in their abdomens. Of the insects kept on kudzu, 30 were male and 32 were female, and 10 of these females (31.3%) were gravid. Thus, the overall

sex ratio was 1.1 males to 1.0 female, and 35% of the females were gravid.

**Foliar Traits of Field Versus Greenhouse Plants.** Percent carbon was significantly higher in field foliage than in greenhouse foliage for both kudzu and soybean (Table 5). Soybean foliage had significantly more carbon than kudzu when plants were grown in the greenhouse but not when plants were grown in the field. Greenhouse soybean had the highest nitrogen content, and greenhouse kudzu had the lowest nitrogen content (Table 5). Total phenolic content was not significantly different for any treatment overall (Table 5), but when analyzed within plant species, field soybean had a significantly higher phenolic content than greenhouse soybean ( $t = 3.01$ ,  $P = 0.0397$ ). Water content was highest for greenhouse kudzu overall (Table 5), and field foliage had significantly lower water content than greenhouse foliage when compared within plant species (kudzu  $t = -6.12$ ,  $P < 0.0001$ ; soybean  $t = -2.52$ ,  $P = 0.0215$ ). Leaves from the field were  $\approx 1.5$  times tougher than leaves from the greenhouse for both kudzu and soybean (Table 5).

For both plant species, field foliage had more than twice the number of trichomes per square millimeter as greenhouse foliage (Table 6; for soybean  $t = 7.38$ ,  $P < 0.0001$ ). Trichome densities for field and greenhouse plants were each  $\approx 17$  times greater on kudzu than soybean. Field-grown kudzu foliage had longer trichomes than greenhouse-grown foliage ( $t = 3.13$ ,  $P = 0.0049$ ; Table 6), and the reverse relationship was true for soybean ( $t = -3.55$ ,  $P = 0.0019$ ).

**Ornaticides trifidus Adult No-Choice Tests.** Adult weevils produced long, narrow feeding scars on kudzu terminals and at the base of trifoliolate leaves or occasionally at the base of individual kudzu leaflets. This damage caused wilting of terminals and death of leaves or leaflets. Adults were frequently observed mating, and several potted kudzu plants and detached kudzu terminals had spiral notches, which have been observed as egg-laying sites in China. However, any eggs or larvae that may have been produced in quarantine did not survive to create the characteristic large galls observed on field plants in the insect's native range (Tayutivutikul and Kusigemati 1992b). Adults died off gradually during the course of the summer: of the 107

**Table 4.** Reproductive output (means  $\pm$  SEM) of *G. tredecimmaculata* reared in 2004, overwintered, and kept on one host plant and foliage type from 16 June to 11 July 2005

Host plant	Foliage type	N <sup>a</sup>	No. offspring	Range <sup>b</sup>	Percent reproducing (N)
Kudzu	Field	24	3.6 $\pm$ 3.58	0-86	4.2 (1)
	Greenhouse	25	1.2 $\pm$ 1.12	0-28	8.0 (2)
Soybean	Field	27	0.6 $\pm$ 0.41	0-9	7.4 (2)
	Greenhouse	23	4.7 $\pm$ 4.61	0-106	8.7 (2)
F			0.46		
df			3,95		
P			0.7104		

<sup>a</sup> N, number of rearing containers with mating pairs of adults.

<sup>b</sup> Minimum and maximum number of larvae produced during the 26-d period for each mating pair.

Table 5. Leaf traits (means  $\pm$  SEM) of kudzu and soybean grown in the field and greenhouse

Plant species	Growing conditions	Percent carbon <sup>a</sup>	Percent nitrogen <sup>a</sup>	Total phenolic content (mg/ml)	Percent water <sup>a</sup>	Leaf toughness (g)
Kudzu	Field	48.7 $\pm$ 0.02a	5.0 $\pm$ 0.03c	0.39 $\pm$ 0.051a	79.9 $\pm$ 0.49b	57.6 $\pm$ 1.41a
	Greenhouse	44.1 $\pm$ 0.10c	4.6 $\pm$ 0.02d	0.33 $\pm$ 0.009a	84.2 $\pm$ 0.51a <sup>b</sup>	39.8 $\pm$ 1.95b
Soybean	Field	48.7 $\pm$ 0.03a	5.6 $\pm$ 0.01b	0.49 $\pm$ 0.050a	78.7 $\pm$ 0.74b	56.0 $\pm$ 3.28a
	Greenhouse	48.2 $\pm$ 0.02b	6.2 $\pm$ 0.01a	0.34 $\pm$ 0.007a <sup>b</sup>	50.9 $\pm$ 0.42b <sup>b</sup>	33.0 $\pm$ 1.28b
F		1640.57	1420.45	4.11	18.93	32.25
df		3,36	3,36	3,8	3,36	3,116
P		<0.0001	<0.0001	<0.0487	<0.0001	<0.0001

Means within a column followed by the same letter are not significantly different ( $P < 0.05$ ; ANOVA, Tukey's studentized range).

<sup>a</sup> Analysis conducted on arcsine-transformed data; original mean percent is shown.

<sup>b</sup> *t*-Test indicates significant difference within plant species.

live adults received in mid-June, about one half (49) remained alive by mid-July. Experiments were terminated on 31 August, with nine weevils still alive (subsequently kept as voucher specimens).

In no-choice tests, all kudzu terminals and potted plants were severely damaged and wilted during each trial after 5-d exposure to 10 weevils per cage (Table 7). Fewer weevils were found on soybean plants ( $1.7 \pm 0.3$ ,  $N = 3$ ) than on kudzu foliage ( $5.7 \pm 0.9$ ,  $N = 3$ ) in the no-choice trial after 1 d. After 5 d, however, all three soybean plants appeared dead, with all leaves chewed at the petiole and hanging wilted and dried from the plant stem. Similar results were obtained for hog-peanut ( $N = 2$ ), with both plants severely damaged and apparently dead after 5 d. Of the other plants tested, a single wilted leaf was observed on one of the bush bean plants, one of the pigeonpea plants, and one of the hyacinth-bean plants ("minor" feeding damage; Table 7). Jack-bean and *E. abyssinica* plants had small holes in their stems, indicating feeding attempts, but no wilted leaves. No feeding was observed on cowpea, alfalfa, or peas. The plants that were severely damaged by the weevils were in the subtribe Glycininae of the tribe Phaseoleae, whereas the other plants tested were from various other subtribes or tribes within the subfamily Faboideae (Table 7).

When groups of 10 *O. trifidus* weevils were given a choice of a soybean plant or a bouquet of kudzu terminals ( $N = 3$ ), there was no damage to any of the soybean plants after 2 d, whereas kudzu terminals were extensively damaged. However, after 5 d, one

soybean plant stem had been chewed through (killing the plant), one had a single wilted trifoliolate leaf, and one had feeding scars visible on some petioles, but not enough to kill any leaves. All kudzu terminals were severely damaged, including those added to the cage on day 3.

## Discussion

*Gonioctena tredecimmaculata* females in quarantine were ovoviviparous, as has been reported for several other species in the genus (Takizawa 1976, Kudo et al. 1995). The beetles that we obtained from China actively consumed foliage and reproduced all summer, but their offspring did not mate or reproduce that year. A similar univoltine life cycle has been reported for other *Gonioctena* spp., with newly emerged beetles feeding for a brief time before entering the soil for diapause and mating and reproducing the following

Table 7. Plant damage due to feeding by *O. trifidus* adults (10 per plant) in 5-d no-choice host specificity tests

Host plant <sup>a</sup>	Tribe, subtribe <sup>b</sup>	Feeding damage <sup>c</sup>
<i>Pueraria montana</i> (kudzu)	Phaseoleae, Glycininae	Severe
<i>Glycine max</i> (soybean)	Phaseoleae, Glycininae	Severe
<i>Amphicarpa bracteata</i> (hog-peanut)	Phaseoleae, Glycininae	Severe
<i>Phaseolus vulgaris</i> (bush bean)	Phaseoleae, Phaseolinae	Minor
<i>Lablab purpureus</i> (hyacinth-bean)	Phaseoleae, Phaseolinae	Minor
<i>Cajanus cajan</i> (pigeonpea)	Phaseoleae, Cajaninae	Minor
<i>Canavalia ensiformis</i> (jack-bean)	Phaseoleae, Diocleinae	None <sup>d</sup>
<i>Erythrina abyssinica</i>	Phaseoleae, Erythrinae	None <sup>d</sup>
<i>Vigna unguiculata</i> (cowpea)	Phaseoleae, Phaseolinae	None
<i>Medicago sativa</i> (alfalfa)	Trifolieae	None
<i>Pisum sativum</i> (peas)	Fabaeae	None

<sup>a</sup> Three soybean and two of all other host plants were tested; two or three cages with kudzu plants or bouquets of fresh terminals were included during each trial.

<sup>b</sup> Based on USDA-GRIN (2006). All plants are in the family Fabaceae (Leguminosae), subfamily Faboideae.

<sup>c</sup> Severe, all plants badly damaged or dead; minor, one wilted leaf on one plant, other plant undamaged; none<sup>d</sup>, small holes in stems but no wilted leaves; none, no damage.

Table 6. Trichome density and length (means  $\pm$  SEM) from kudzu and soybean foliage grown in the field and greenhouse

Plant species	Growing conditions	N	No. of trichomes per mm <sup>2</sup>	Length of trichomes <sup>a</sup> (mm)
Kudzu	Field	12	60.1 $\pm$ 4.31a	0.4 $\pm$ 0.05a
	Greenhouse	12	26.3 $\pm$ 3.24 <sup>b</sup>	0.2 $\pm$ 0.01b <sup>b</sup>
Soybean	Field	12	3.5 $\pm$ 0.21c	0.3 $\pm$ 0.02ab
	Greenhouse	11	1.5 $\pm$ 0.15c <sup>b</sup>	0.4 $\pm$ 0.02a <sup>b</sup>
F			97.76	8.21
df			3,43	3,43
P			<0.0001	0.0002

Means within a column followed by the same letter are not significantly different ( $P < 0.05$ ; ANOVA, Tukey's studentized range test).

<sup>a</sup> Average length of 10 trichomes per sample.

<sup>b</sup> *t*-Test indicates significant difference within plant species.

year (Waloff and Richards 1958, Takizawa 1976, Mason and Lawson 1982, Mardulyn et al. 1997).

The fact that <10% of the overwintered *G. tredecimmaculata* adults that we kept as pairs in plastic tubs reproduced in 2005 (the second year in quarantine) suggests that these beetles may not have had adequate opportunities to mate or that conditions were not conducive for mating or oviposition. Similar results were reported by Mason and Lawson (1982), who stated that unmated *Gonioctena americana* (Schaeffer) did not larviposit and that laboratory-reared pairs held in small containers did not produce offspring. Of the females in our study that failed to reproduce the second year, 35% were nevertheless found to be gravid when they were dissected in the fall.

In no-choice tests, *G. tredecimmaculata* larvae fed on soybean, hog-peanut, and kudzu, with 21, 50, and 64%, respectively, surviving to adulthood. The fact that actively reproducing beetles from China burrowed into the soil when they were placed on soybean, whereas beetles kept on kudzu continued to reproduce, suggests that beetles feeding on kudzu might not accept soybean under open-field conditions. For the adults that did reproduce in 2005 in our tests, however, equivalent oviposition was observed on kudzu and soybean. The physiological host range (Sheppard et al. 2005) of *G. tredecimmaculata* thus seems to include soybean and the native woodland plant, hog-peanut, as well as kudzu, because all three plant species supported growth and development of at least some larvae through to the adult stage, and soybean, at least, was acceptable for oviposition.

Field-grown foliage of kudzu and soybean had higher total carbon content, greater leaf toughness, higher trichome density, and lower water content than greenhouse-grown foliage. Field soybean foliage also had a higher total phenolic content than greenhouse soybean foliage, but no differences were found for kudzu. Significant differences were detected for trichome length and total nitrogen content between growing conditions, but no overall patterns in field versus greenhouse or kudzu versus soybean comparisons were evident for these traits. Despite the differences found between field- and greenhouse-grown kudzu and soybean, however, there were no consistent differences in *G. tredecimmaculata* feeding, survival, or host choice. This insect is thus able to cope with variations in leaf traits such as total carbon and nitrogen content, toughness, water content, total phenolic content, and trichome density and length, with no apparent cost to development or survival.

Far less information was obtained on the biology and host range of *O. trifidus*, because we were unable to rear it through from egg to adult on detached kudzu terminals or on young potted plants in quarantine. However, this species also fed as an adult on the same two nontarget species as *G. tredecimmaculata*, soybean and hog-peanut, causing extensive damage and even death of plants in no-choice tests. This insect likely cannot complete its life cycle on soybean or hog-peanut, because its larvae feed internally and cause stem galls on kudzu. Stem-feeding insects gen-

erally have a close association with their plant host, including host phenology (Frenzel and Brandl 1998). The fact that kudzu is a perennial whereas soybean is an annual plant also suggests that this gall-maker would have a difficult time reproducing on soybean. Although hog-peanut is a perennial plant, its stems are far thinner than those of kudzu, making it doubtful that it could support *O. trifidus* larval growth.

Choice tests suggested a preference for kudzu over soybean for both *G. tredecimmaculata* and *O. trifidus*, but soybean plants were damaged by both insect species even when kudzu was present. Soybean and hog-peanut were the only plant species tested that were from the same subtribe of the Phaseoleae as kudzu (Glycininae). Thus, feeding on these species by *G. tredecimmaculata* and *O. trifidus* is in accordance with the phylogenetic approach of Wapshere (1974) to host plant testing, which states that species closely related to the target weed are at a greater risk of attack than more distantly related species.

Weed biological control scientists generally agree that no-choice tests are a necessary first step in evaluating potential agents. However, further tests may be necessary when results are ambiguous or where "false-positive" results are possible (Cullen 1989, Marohasy 1998, Briese 2005). Such "false positives" can lead to the rejection of an agent that would otherwise be safe for introduction, and this has led some authors to argue that the decision to reject an agent should not be based solely on laboratory studies (Cullen 1989, Balciunas et al. 1996) but should include open-field choice tests to assess the realized host range of an agent. For example, no-choice host range tests of *Ceratapion basicorne* (Illiger) (Coleoptera: Apionidae) in the laboratory showed that this insect could oviposit and complete its development on the nontarget, agriculturally important safflower (*Carthamus tinctorius* L., Asteraceae: Cardueae) in addition to its target host, yellow starthistle (*Centaurea solstitialis* L., Asteraceae: Cardueae). Under field conditions, however, *C. basicorne* did not attack safflower even when 100% of nearby yellow starthistle plants were infested (Smith et al. 2006). Clement and Cristofaro (1995) cited several additional examples of insects that were originally rejected as biological control agents because they fed on crop species in the laboratory but were later shown not to attack these species in the field.

To our knowledge, neither *G. tredecimmaculata* nor *O. trifidus* has been reported as a pest of soybean in China, and neither species has been observed on soybean growing near kudzu in China (J.H.S., unpublished data). Thus, the next step with these species could be open-field testing in China to further understand their field or realized host range. However, the high economic importance of soybean will make it difficult to justify importing these insects for biological control of kudzu unless unequivocal evidence is produced that shows the risk of an expanded host range to be extremely low or nonexistent.

## Acknowledgments

We thank B. Vasilas, D. Tallamy, and M. Smith for help and suggestions throughout the study and L. Ertle, R. Fuester, and K. Swan of the USDA-ARS laboratory for providing advice and space in quarantine and assisting with the permitting process. R. Shockley provided access to his kudzu patch in Fair Hill, MD. F. Halaweish, South Dakota State University, provided the phenolic analysis protocol, and C. Keil helped with carrying it out. C. Olsen, W. Honaker, C. Golt, and C. Davies assisted with the leaf trait analyses. We thank R. Beardon of the USDA Forest Service for supporting this research. Additional financial support came from the Southern Research Station Research Work Unit 4505, "Insects and Diseases of Southern Forests."

## References Cited

- Alonso-Zaragoza, M. A., and C.H.C. Lyal. 1999. A world catalogue of families and genera of Curculionioidea (Insecta: Coleoptera) (excepting Scolytidae and Platypodidae). Entomopraxis, Barcelona, Spain.
- Balcunas, J. K., D. W. Burrows, and M. F. Purcell. 1996. Comparison of the physiological and realized host-ranges of a biological control agent from Australia for the control of the aquatic weed, *Hydrilla verticillata*. Biol. Control 7: 148-158.
- Bezděk, J. 2002. A review of the *Gonioctena tredecimmaculata* (Jacoby, 1888) group (Coleoptera, Chrysomelidae, Chrysomelinae). Entomol. Basiliensis 24: 7-22.
- Blackwell, J. 1973. The vine that ate the south. Plants Gard. Brooklyn Bot. Gard. Rec. 30: 29-30.
- Bonsi, C., E. Rhoden, A. Woldegehebriel, P. Mount, S. Solaiman, R. Noble, G. Paris, C. McMahon, H. Pearson, and B. Cash. 1991. Kudzu-goat interactions—a pilot study, pp. 84-88. In S. G. Solaiman and W. A. Hill (eds.), Using goats to manage forest vegetation: a regional inquiry. Tuskegee Univ. Agric. Exp. Sta., Tuskegee, AL.
- Breider, E. V. 1961. Control of honeysuckle and kudzu. U.S. Department of Agriculture Forest Service, Southeast Forest Experiment Station, Asheville, NC.
- Briese, D. T. 2005. Translating host-specificity test results into the real world: the need to harmonize the yin and yang of current testing procedures. Biol. Control 35: 208-214.
- Britton, K. O., D. Orr, and J. Sun. 2002. Kudzu, pp. 325-330. In R. G. Van Driesche, S. Lyon, B. Blossey, M. S. Hoddle, and R. Beardon (eds.), Biological control of invasive plants in the Eastern United States. U.S. Department of Agriculture Forest Service, Morgantown, WV.
- Bruneau, A., J. J. Doyle, and J. L. Doyle. 1994. Phylogenetic relationships in *Phaseoleae*: evidence from chloroplast DNA restriction site characters, pp. 309-330. In M. Crisp and J. J. Doyle (eds.), Advances in legume systematics. 7: phylogeny. Royal Botanic Gardens, Kew, UK.
- Cipollini, D. F., Jr. 1997. Wind-induced mechanical stimulation increases pest resistance in common bean. Oecologia (Berl.) 111: 84-90.
- Clement, S. L., and M. Cristofaro. 1995. Open-field tests in host-specificity determination of insects for biological control of weeds. Biocontrol Sci. Technol. 5: 395-406.
- Cullen, J. M. 1989. Current problems in host-specificity screening, pp. 27-36. In E. S. Delfosse (ed.), Proceedings of the VII international symposium on biological control of weeds. Istituto Sperimentale per la Patologia Vegetale, Rome, Italy.
- Everest, J. W., J. H. Miller, D. M. Ball, and M. G. Patterson. 1991. Kudzu in Alabama: history, uses, and control. Alabama Cooperative Extension, Auburn, AL.
- Forseth, I. N., Jr., and A. F. Innis. 2004. Kudzu (*Pueraria montana*): history, physiology, and ecology combine to make a major ecosystem threat. Crit. Rev. Plant Sci. 23: 401-413.
- Frenzel, M., and R. Brandl. 1998. Diversity and composition of phytophagous insect guilds on Brassicaceae. Oecologia (Berl.) 113: 391-399.
- Frye, M. J. 2006. Biology and host specificity of *Gonioctena tredecimmaculata* (Coleoptera: Chrysomelidae): a potential biological control agent for kudzu. MS thesis, University of Delaware, Newark, DE.
- Harrington, T. B., L. T. Rader-Dixon, and J. W. Taylor, Jr. 2003. Kudzu (*Pueraria montana*) community responses to herbicides, burning, and higher-density loblolly pine. Weed Sci. 51: 965-974.
- Jacoby, M. 1888. Descriptions of new species of phytophagous Coleoptera from Kiukiang (China). Proc. Zool. Soc. Lond. 1888: 339-351.
- Kajita, T., H. Ohashi, Y. Tateishi, C. D. Bailey, and J. J. Doyle. 2001. rbcL and legume phylogeny, with particular reference to Phaseoleae, Millettieae, and allies. Syst. Bot. 26: 515-536.
- Kudo, G. 2003. Variations in leaf traits and susceptibility to insect herbivory within a *Salix miyabeana* population under field conditions. Plant Ecol. 169: 61-69.
- Kudo, S., E. Ishibashi, and S. Makino. 1995. Reproductive and subsocial behaviour in the ovoviparous leaf beetle *Gonioctena sibirica* (Coleoptera: Chrysomelidae). Ecol. Entomol. 20: 367-373.
- Marduly, P., M. C. Milinkovitch, and J. M. Pasteels. 1997. Phylogenetic analyses of DNA and allozyme data suggest that *Gonioctena* leaf beetles (Coleoptera: Chrysomelidae) experienced convergent evolution in their history of host-plant family shifts. Syst. Biol. 46: 722-747.
- Marohasy, J. 1998. The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour. Biocontrol News Inform. 19: 13N-20N.
- Mason, M. L., and F. A. Lawson. 1982. Biology of the American aspen beetle (Coleoptera: Chrysomelidae: *Gonioctena americana* [Schaeffer]) in the Medicine Bow National Forest, Wyoming. J. Kans. Entomol. Soc. 55: 779-788.
- McWilliams, D. A., D. R. Berglund, and G. J. Eudres. 1999. Soybean growth and management quick guide. NDSU Extension Service, Fargo, ND.
- Mitich, L. W. 2000. Intriguing world of weeds: kudzu (*Pueraria lobata* (Willd.) Ohwi). Weed Technol. 14: 231-235.
- Munger, C. T. 2002. *Pueraria montana* var. *lobata*. Fire effects information system (<http://www.fs.fed.us/database/feis/plants/vine/puemon1/introductory.html>).
- Pajni, H. R., and S. Dhir. 1987. A generic revision of the subfamily Alcidodinae with the proposal of seven genera (Alcidodinae; Curculionidae; Coleoptera). Res. Bull. (Science) Panjab Univ. 38: 27-34.
- Perez-Hernandez, O., X. B. Yang, S. Pivonia, and J. Guan. 1994. Assessing the role of kudzu *Pueraria lobata* in potential epidemics of soybean rust in the United States. Phytopathology 94: S83.
- Pivonia, S., and X. B. Yang. 2004. Assessment of the potential year-round establishment of soybean rust throughout the world. Plant Dis. 88: 523-529.
- Sheppard, A. A., R. D. van Klinken, and T. H. Heard. 2005. Scientific advances in the analysis of direct risks of weed

- biological control agents to nontarget plants. *Biol. Control* 35: 215-226.
- Shurtleff, W., and A. Aoyagi. 1985. *The book of kudzu: a culinary & healing guide*. Avery Publishing Group, New York.
- Smith, L., R. Hayat, M. Cristofaro, C. Tronci, G. Tozlu, and F. Lecce. 2006. Assessment of risk of attack to safflower by *Ceratopion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of *Centaurea solstitialis* (Asteraceae). *Biol. Control* 36: 337-344.
- Snedecor, G. W., and W. G. Cochran. 1967. *Statistical methods*, 6th ed. Iowa State University Press, Ames, IA.
- Sun, J., Z. Liu, K. O. Britton, P. Cai, D. Orr, and J. Hough-Goldstein. 2006. Survey of phytophagous insects in China for a biocontrol perspective on kudzu, *Pueraria montana* var. *lobata* (Willd.) Maesen and S. Almeida (Fabaceae). *Biol. Control* 36: 22-31.
- Susko, D. J., J. P. Mueller, and J. F. Spears. 2001. An evaluation of methods for breaking seed dormancy in kudzu (*Pueraria lobata*). *Can. J. Botany* 79: 197-203.
- Tabor, P., and A. W. Susott. 1941. Zero to thirty million mile-a-minute seedlings. *Soil Conserv.* 7: 61-65.
- Takizawa, H. 1976. Larvae of the genus *Conioctena* Chevrolat (Coleoptera, Chrysomelidae): descriptions of Japanese species and the implications of larval characters for the phylogeny. *Kontyû* (Tokyo) 44: 444-468.
- Tayutivutikul, J., and K. Kusigemati. 1992a. Biological studies of insects feeding on the kudzu plant, *Pueraria lobata* (Leguminosae) I. List of feeding species. *Mem. Fac. Agr. Kagoshima Univ.* 28: 89-124.
- Tayutivutikul, J., and K. Kusigemati. 1992b. Biological studies of insects feeding on the kudzu plant, *Pueraria lobata* (Leguminosae) II. Seasonal abundance, habitat and development. *South Pacific Study* 13: 37-90.
- Turnipseed, S. G., and M. Kogan. 1976. Soybean entomology. *Annu. Rev. Entomol.* 21: 247-282.
- Underwood, N., N. Rausher, and W. Cook. 2002. Bioassay versus chemical assay: measuring the impact of induced and constitutive resistance on herbivores in the field. *Oecologia* (Berl.) 131: 211-219.
- U.S. Department of Agriculture-Germplasm Resources Information Network [USDA-GRIN]. 2006. U.S. Department of Agriculture, Agricultural Research Service, Germplasm Resources Information Network (<http://www.ars-grin.gov>).
- Viviani, T., L. Conte, G. Cristofolini, and M. Speranza. 1991. Sero-systematic and taximetric studies on the Phaseoleae (Fabaceae) and related tribes. *Bot. J. Linn. Soc.* 105: 113-136.
- Waloff, N., and O. W. Richards. 1958. The biology of the Chrysomelid beetle, *Phytodecta olivacea* (Forster) (Coleoptera: Chrysomelidae). *Trans. R. Entomol. Soc. Lond.* 110: 99-116.
- Wapshere, A. J. 1974. A strategy for evaluating the safety of organisms for biological weed control. *Ann. Appl. Biol.* 77: 201-211.
- Witt, A.B.R., A. J. McConnachie, and R. Stals. 2004. *Alcidodes sedi* (Col.: Curculionidae), a natural enemy of *Bryophyllum delagoense* (Crassulaceae) in South Africa and a possible candidate agent for the biological control of this weed in Australia. *Biol. Control* 31: 380-387.
- Woodhead, S. 1981. Environmental and biotic factors affecting the phenolic content of different cultivars of *Sorghum bicolor*. *J. Chem. Ecol.* 7: 1035-1047.
- Zheng, H., Y. Wu, J. Ding, D. Binion, W. Fu, and R. Reardon. 2004. Invasive plants of Asian origin established in the United States and their natural enemies, vol. 1. U.S. Department of Agriculture Forest Service, Morgantown, WV.
- Zheng, H., Y. Wu, J. Ding, D. Binion, W. Fu, and R. Reardon. 2005. Invasive plants established in the United States that are found in Asia and their associated natural enemies, vol. 2. U.S. Department of Agriculture, Morgantown, WV.

Received for publication 15 March 2007; accepted 29 August 2007.

