N O T E

Population Census of *Megacopta cribraria* (Hemiptera: Plataspidae) in Kudzu in Georgia, USA, 2013–2016[1]

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Since its invasion of the southeastern United States in 2009, the bean plataspid or kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), has become an agricultural pest of soybean, Glycine max (L.) Merrill (Ruberson et al. 2013, J. Appl. Zool. Entomol. 48: 3–13; Gardner et al. 2013, J. Entomol. Sci. 48: 355–359), initiating studies to determine potential biological control agents. Surveys of kudzu, *Pueraria montana* var. *lobata* (Willd.) Maesen & S. Almeida, in the more northern counties of Georgia in 2010, 2011, and 2012 found no parasitized kudzu bug egg masses and also concluded that occasional generalist predators and pathogens did not appear to effectively control this species (Ruberson et al. 2013; Zhang et al. 2012, Environ Entomol. 41:40–50). However, in 2013, an egg parasitoid identified as *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae) by Walker A. Jones (USDA-ARS) and confirmed by Elijah J. Talamas (USDA, Smithsonian Institution, Washington, DC) was found in kudzu bug eggs in kudzu initially in north-central Georgia, where parasitism rates ranged from 47.5% to 95% (Gardner et al. 2013). This parasitoid was subsequently reported from kudzu bug eggs in Alabama, South Carolina, Mississippi (Gardner et al. 2013), southern Georgia (P. Roberts and D.M.O. pers. obs.), and Florida (Medal et al. 2015, Fla. Entomol. 98: 1250–1251). However, since its initial discovery in 2013, the parasitoid has only been found in southern Georgia and Florida, presumably because of natural mortality factors impacting its ability to overwinter in the more northern areas of its reported range.

Epizootics of the entomogenous fungus *Beauveria bassiana* (Balsamo) Vuillemin were also observed and confirmed in soybean fields, kudzu patches, or both in several locations in South Carolina (pers. commun. D. Greene and J. Greene, Clemson University), and North Carolina (pers. commun., E. Scocco Niland, Wingate University). High mortality rates of overwintering kudzu bug adults

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resulting from infection by the fungus were subsequently reported in South Carolina (J. Greene) and Tennessee (L. Faust, University of Tennessee, Knoxville).

Our objectives in the survey reported herein were to (a) assess infection levels of *B. bassiana* in populations of overwintering adult kudzu bugs in selected kudzu patches in southern and north-central Georgia in 2016, and (b) summarize data on the occurrence of kudzu bug and natural enemy populations collected from selected areas from 2013 through 2016.

Four survey sites were selected in southern Georgia and were located in Tift, Turner, Dooly, and Houston counties. Each site comprised an area $\geq$0.4 ha and were separated from one another by at least 16 km. Sampling of the sites was conducted from 17 April to 12 June in 2013, 16 April to 9 July in 2014, 4 April to 19 May in 2015, and 21 March to 16 May in 2016. At these southern Georgia sites, adults and nymphs were enumerated on the sampling date by taking a 20-sweep sample per site within 10 m of the patch edge. Sweep samples were transferred to plastic bags, returned to the laboratory, and frozen. Numbers of kudzu bug adults, nymphs (1st to 5th instars), and any natural enemies in the sample were identified, counted, and recorded after thawing the samples.

Within these sites, the terminal growth (1 m in length) of 25 randomly selected kudzu vines (where most *M. cribraria* eggs are oviposited) within 10 m of the patch edge were cut and placed in individual bags for transport to the laboratory. In the laboratory, egg masses were counted and the foliage with the individual egg masses was removed, placed in containers, and incubated for 2 weeks to determine status as “parasitized,” “normal eclosion,” or “infertile or embryo mortality.” If parasitized, the parasitoid was collected and identified.

Three sites also were established in kudzu in north-central Georgia and were located in Spalding, Clayton, and Walton counties. Each site was also comprised of at least 0.4 ha of kudzu. These sites were monitored at weekly intervals from mid-April to early July each year as described above. Collected masses were enumerated and placed individually in 2-dram glass vials that were plugged with cotton, incubated for 2 weeks at 23°C, and then assessed for parasitism as previously described. Sweep samples were frozen and eventually thawed to enumerate numbers of nymphs and adults collected.

In February 2016, overwintering adults were sampled in the soil under kudzu vines and under the bark of nearby trees at all seven sites. All debris on the soil surface for each sample was carefully removed from the surface, and the soil at the surface was sifted by hand for dead or overwintering adults. A total of area of 0.25 m^2 of the soil was sampled from 15 randomly selected locations at each site, and all adults collected were counted and recorded as infected or not infected with *B. bassiana*. For the samples obtained from surrounding trees, bark was removed from areas of the tree until at least one kudzu bug was found. All adults observed were counted and recorded as either infected or not infected with *B. bassiana*. Three to five sites on each tree were sampled until at least 15 samples were obtained. Characteristic white hyphal emergence and sporulation on kudzu bug cadavers served as the indicator of infection by *B. bassiana*. Randomly selected cadavers were returned to the laboratory, where identification of the fungus was confirmed by microscopic examination.

The effect of year on the density of eggs, nymphs, and adults was tested with ANOVA using Tukey’s HSD to separate significantly different treatment means

Table 1. Mean (± SE) cumulative numbers of adults, nymphs, and predators per sweep and eggs per stem of *M. cribraria* in kudzu in southern and north-central Georgia, 2013–2016.*

	2013	2014	2015	2016
South Georgia sites				
Adult	114.84 ± 19.22 a	50.67 ± 8.09 b	47.94 ± 16.55 b	7.29 ± 2.01 c
Nymph	155.06 ± 52.88 a	33.12 ± 7.97 b	0.50 ± 0.36 c	1.53 ± 0.80 c
Egg	49.79 ± 2.42 a	13.51 ± 0.62 b	20.70 ± 4.57 b	3.17 ± 0.28 c
Predators	9.00 ± 1.55 a	4.20 ± 0.55 b	2.89 ± 0.74 c	4.45 ± 0.58 b
North-central Georgia sites				
Adult	33.26 ± 8.01 a	14.52 ± 0.72 b	13.53 ± 0.65 b	0.12 ± 0.10 c
Nymph	39.62 ± 8.45 a	8.31 ± 2.32 b	0.13 ± 0.13 c	0.00 ± 0.00 c
Egg	33.76 ± 7.29 a	23.12 ± 9.21 b	14.28 ± 1.67 b	0.09 ± 0.12 c

* Means within a row followed by the same lowercase letter are not significantly different (ANOVA; Tukey's HSD; $P < 0.001$).

(SAS Institute Inc. 1998, Cary, NC). Chi-square analyses were used to test for kudzu bug fungal infection rates among sites and location within sites (soil and under bark of trees) (SAS Institute Inc. 1998).

Cumulative numbers of adults, nymphs, and eggs collected and pooled over all four south Georgia sites significantly decreased over the successive years (2013–2016) of this study ($F = 13.80$, df $= 3$, $P < 0.001$ for adults; $F = 7.61$, df $= 3$, $P < 0.001$ for nymphs; $F = 135.92$, df $= 3$, $P < 0.001$ for eggs) (Table 1). A similar significant decline in cumulative numbers of adults, nymphs, and eggs was observed over the four years at the three north-central Georgia sites ($F = 23.71$, df $= 3$, $P < 0.001$ for adults; $F = 12.74$, df $= 3$, $P < 0.001$ for nymphs; $F = 6.38$, df $= 3$, $P < 0.001$ for eggs) (Table 1).

Significant differences in cumulative numbers of adults, nymphs, and eggs also occurred across the four sites in south Georgia. Mean (± SE) numbers of adults by location were Dooly Co. (92.89 ± 19.27) > Houston Co. (58.97 ± 11.95) > Tift Co. (30.67 ± 7.19) = Turner Co. (30.08 ± 5.74). Numbers of nymphs were Houston Co. (85.69 ± 35.19) = Dooly Co. (63.22 ± 34) = Tift Co. (32.00 ± 16.69) > Turner Co. (10.38 ± 3.93). Cumulative density of eggs by site were Dooly Co. (39.08 ± 2.14) > Houston Co. (23.53 ± 2.55) = Turner Co. (7.22 ± 0.61) = Tift Co. (8.31 ± 0.63). Predator density (primarily spiders and coccinellids) was low throughout the study, with significantly higher numbers in 2013 compared with other years ($F = 7.18$, df $= 3$, $P < 0.001$) (Table 1). No significant differences among the north-central Georgia sites with respect to kudzu bug life stage were detected. Predator occurrence data were not collected at these three sites.

In 2013 and 2014, *P. saccharalis* was recovered from kudzu bug eggs collected from two to five soybean fields located within 8 km of each of the four south Georgia kudzu sites monitored. The parasitoid was collected from eggs on kudzu at the

USDA Belflower Farm (Tift Co.) in mid-August 2015, but no parasitoids were found to date in 2016. *Paratelenomus saccharalis* parasitism levels observed were 42.5% ($n = 143$ eggs) at the Spalding Co. site, 58% ($n = 198$ eggs) at the Clayton Co. site, and 56.8% ($n = 441$ eggs) at the Walton Co. site in 2013. Only a total of nine parasitoids were found at the north-central Georgia sites in 2014 (five from Spalding Co., four from Clayton Co.) with 1,250 to 1,475 eggs examined at each site. No parasitoids were found in either 2015 or 2016 at these sites.

Total numbers of overwintering adult kudzu bugs recovered from the combined soil and tree samples and confirmed as infected with *B. bassiana* differed among the south Georgia sites ($\chi^2_3 = 88.59$, $P < 0.001$) with percentage infection of 55% at the Tift Co. and Houston Co. sites, 38% at the Dooly Co. site, and 20% at the Turner Co. site. The percentage of adults found infected with the fungus also differed with the substrate (tree versus soil) sampled (Dooly $\chi^2_1 = 44.31$, $P < 0.001$; Houston $\chi^2_1 = 52.88$, $P < 0.001$; Tift $\chi^2_1 = 21.54$, $P < 0.001$; Turner $\chi^2_1 = 189.42$, $P < 0.001$), with significantly higher numbers of adults infected found on trees than in the soil samples. However, the magnitude of those differences varied among the sites (Dooly: 67% from trees, $n = 105$, 33% from soil, $n = 695$; Houston: 98% from trees, $n = 60$, 48% from soil, $n = 322$; Tift: 73% from trees, $n = 51$, 14% from soil, $n = 22$; Turner: 86% from trees, $n = 56$, 2% from soil, $n = 200$). No overwintering adults were recovered from the soil substrate sampling at either the Spalding Co. site, Clayton Co. site, or Walton Co. site. The percentage of cadavers collected from trees and confirmed as being infected with *B. bassiana* was 85% ($n = 42$) at the Spalding Co. site, 96% ($n = 51$) at the Clayton Co. site, and 89% ($n = 54$) at the Walton Co. site.

Populations of *M. cribraria* decreased dramatically over the duration of this census. The egg parasitoid *P. saccharalis*, which was initially discovered in 2013 (Gardner et al. 2013), is thought to have impacted kudzu bug populations initially. Winter kill also likely impacted overwintering populations in January 2014 and January 2015 when temperatures dropped below −14°C at the north-central Georgia sites. Reduced parasitism by *P. saccharalis* after 2013 may also be due to these winter temperatures. Epizootics of the fungus *B. bassiana*, especially in overwintering adult populations, are also contributing to this overall decline of *M. cribraria* in Georgia.

Visual estimation of the kudzu growth at the sites monitored also fluctuated over the duration of the study. Growth was reduced by as much as 80% at some sites over time. Indeed, Zhang et al. (2012) recorded 33% reduction in kudzu biomass from *M. cribraria* feeding. Thus, we saw less kudzu growth with increased amount of emergent weeds and vegetation at all sites as the study progressed. However, when the populations of kudzu bugs declined in 2014 and 2015 in north-central Georgia, we observed resurgence in growth of kudzu at those sites. We anticipate a similar response in growth in south Georgia if populations remain low as thus far seen in 2016 in that region of the state.