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Population Dynamics and Parasitism of the Kudzu Bug, *Megacopta cribraria*, by Egg Parasitoid, *Paratelenomus saccharalis*, in Southeastern USA

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Abstract: *Megacopta cribraria* (Fabricius) (Hemiptera: Plataspidae), commonly called the kudzu bug, is a pest of concern in many soybean and legume-producing states. It was first detected in the United States in 2009. In the southeastern United States, *M. cribraria* reduced crop yields by 47% in untreated soybean fields. *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae) is a known parasitoid of the kudzu bug, and a potential biological control agent. This study was comprised of three phases: (1) Preliminary assessment of the presence of the kudzu bug and its parasites in north Florida and south Georgia. (2) Measurements of the levels of parasitism in 12 sites selected from the preliminary evaluation to compare population dynamics in two growing seasons in agricultural, forested, and urban areas. (3) Laboratory studies to measure parasitism after 21 days in controlled environments. The preliminary assessment showed that in both 2016 and 2017, *P. saccharalis* emerged from eggs of *M. cribraria* collected in Leon and Gadsden County. Additionally, parasitism was recorded for the recently discovered egg parasitoid in north Florida, *Ooencyrtus nezarae* (Ishii) (Hymenoptera: Encyrtidae). In the assessment of parasitism in the field, differences were observed in the average level of parasitism within the urban and forest area in both years, and in the agricultural area for 2017. Between-group comparisons indicated significant differences between the average parasitism levels in agricultural, urban, and forested areas in 2016; in 2017, the differences between the areas were not significant. In the laboratory, after 21 days of observation, an average of 77.4% of freshly laid and 61.6% of cooled eggs were parasitized by *P. saccharalis*, and adult parasitoids emerged. There was a significant difference in the emergence of freshly laid eggs over cooled eggs. This study suggests that both *P. saccharalis* and *O. nezarae* exert some levels of natural control of the kudzu bug in the field and may be useful as biological control agents in an integrated pest management program.

Keywords: urban; forested; agricultural area; kudzu; catnip; generalist; specialist; soybean

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1. Introduction

Megacopta cribraria (Fabricius) (Hemiptera: Plataspidae), commonly called the kudzu bug, is a pest of concern in many soybean (*Glycine max* [L.] Merr.; Fabales: Fabaceae) and legumes producing states [1]. This pest is a native of Asia and was first detected in the United States in 2009 [2]. It has a high reproductive potential and is capable of completing three generations per year [3,4]. *Megacopta cribraria* is known for its capability to reduce the biomass of kudzu [*Pueraria montana* var. *lobata* (Willd.)], its main host, by up to 33% [5]. However, in the southeastern United States, *M. criberia*, a phloem feeder, has been observed primarily on kudzu and soybean [6]. Heavy feeding by nymphs and adults on soybean leaves, petioles, and stems tends to weaken and stress plants, and could result to fewer pods, fewer seeds, and smaller seed size [7]. In addition, intensity of feeding of *M. cribraria* can reduce the assimilation of photoassimilates in legumes by defoliation and excess sooty mold growth on leaves [5]. In the southeastern United States, *M. cribraria* reduced crop yields in untreated fields from 0 to 47%, although the average yield loss was 18% [8,9]. In controlled experimental plots, yield losses reached 59.6% [7].

Three important families of egg parasitoids have been reported on *M. cribraria* in its native range: Platygastridae, Aphelinidae, and Encyrtidae [8]. The Aphelinidae are represented by older records from Malaysia, India, and Zaire [10] under the name *Dirphys boswelli* (Girault) (now *Encarsia*) [11]. Females of *Ooencyrtus nezarae* (Ishii) (Hymenoptera: Encyrtidae) and *Paratelenomus saccharalis* (Hymenoptera: Platygastridae) are commonly observed parasitizing eggs of *M. cribraria* [12]. Records of egg parasitism on *M. cribraria* were also reported from soybeans in other provinces of China (e.g., Jiangsu and Fujian) by both *P. saccharalis* and encyrtid species of *Ooencyrtus* (mainly *O. nezarae* Ishii). In those locations, parasitism was observed from May to October at levels ranging from 22.4% to 76.9% [8,13]. *Ooencyrtus* sp. is widely distributed in Korea, Japan, China, Brazil, and Thailand [14,15], and is also reported in North America [16].

Unlike *P. saccharalis, O. nezarae* parasitizes eggs of 13 hemipteran species [15], including Pentatomidae, Coreidae, and Alydidae [16]. Parasitism of *M. cirbraria* on kudzu and catnip (*Nepeta cataria*) by *O. nezarae* was first reported in Florida by Diedrick et al. [17] (Figure 1). In the United States, few studies have evaluated the level of parasitism of egg masses of the kudzu bug by this egg parasitoid [3,17,18]. Generally, egg parasitoids are good candidates for biological control of various stink bugs and have been used successfully in augmentation (the release of additional numbers of a natural enemy) and importation (the deliberate introduction of an exotic natural enemy) approaches [19,20].

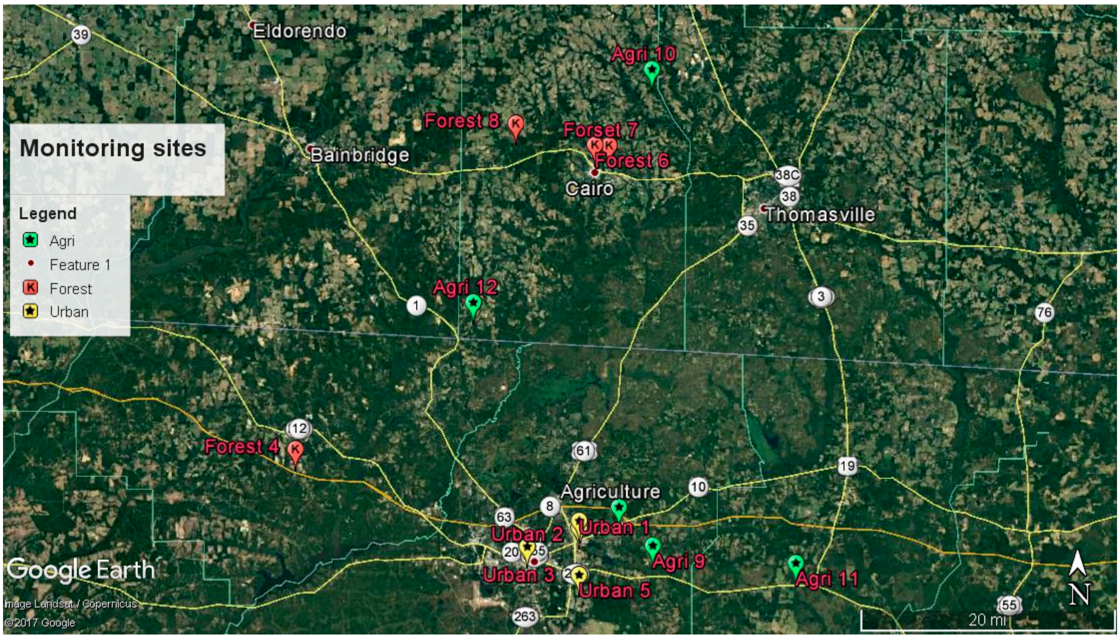

Figure 1. Map showing monitoring sites for *Paratelenomus saccharalis* in north Florida and south Georgia, 2016 and 2017.

This paper reports the population dynamics of the kudzu bug and its parasites in relation to precipitation in north Florida and South Georgia. Precipitation patterns, and how they are affected by climate change, will have profound effects on the population dynamics of both herbivorous insects and their natural enemies [21,22]. Parasitism of the recently discovered parasitoid in north Florida, *O. nezarae*, was compared to that of *P. saccharalis*, a known specialist of the Plataspidae. Additionally, we assessed the level of egg parasitism of *P. saccharalis* on *M. cribraria* in laboratory and field experiments, and compared parasitism of *P. saccharalis* against *Ooencyrtus nezarae*, which was found in a forested site at the end of the second year of these studies.

2. Materials and Methods

2.1. Preliminary Evaluation of the Distribution on P. saccharalis in North Florida and South Georgia

Eggs of *M. cribraria* were collected biweekly in the field from 23 March to 25 September 2016, and from 27 March to 13 August 2017, in four counties: Leon, Gadsden, and Jefferson counties in north Florida, and Grady County in south Georgia. The egg masses were attached predominantly to shoot tips or leaves of host plants. Eggs masses on foliage were gathered from urban, forested, and agricultural areas at different sites in each county. The collected egg masses were placed in plastic containers (15.24 cm × 15.24 cm) with ventilated covers, then put in a cooler with ice packs and transported to the laboratory of the Center for Biological Control (CBC), Florida Agricultural and Mechanical University (FAMU). The plastic containers were placed in rearing cages (60 cm × 60 cm × 60 cm, Bug Dorm II, BioQuip Products) and kept at ambient temperature (27 ± 1 °C) until the emergence of kudzu bugs or their parasitoids. The *M. cribraria* eggs were observed for emergent adults of *P. saccharalis* for up to 21 days, a period based on data of [23]. Prevalence was expressed as a binary variable in a yes/no format based on whether or not the eggs were parasitized by *P. saccharalis.* Precipitation and temperature data were obtained from US Climate Data 2022 (version 3.0) by US Climate Data.

2.2. Assessment of Parasitism in the Field

From the areas surveyed for the presence of *P. saccharalis* in the preliminary evaluation, 12 sites were selected to assess the parasitism level of *P. saccharalis* on *M. cribraria* (Figure 1). Twenty-five plants were marked at each site in the urban, forested, and agricultural areas. This resulted in five replications per site. All replications were marked with flags so that the same plants could be followed during the season. The host plants were kudzu and soybean in the agricultural area, and kudzu in the forested and urban area. For soybean, five adjacent host plants were chosen for monitoring; for kudzu, five plant areas of 929 cm^2 (1 sq ft) each were delimited using a quadrant assembled with 4 sections of 30.48 cm polyvinyl chloride (PVC) pipes.

Egg masses of *M. cribraria* from all marked plants were collected and counted. All adults and nymphs of *M. cribraria* observed were also counted. The egg masses were transported to the CBC laboratory as previously described. In the laboratory, the number of eggs were counted using an Olympus stereoscope. Individual egg masses were then placed in separate glass vials (1.7 cm × 6 cm), capped with absorbent cotton, and placed in observation cages (30 cm × 30 cm × 30 cm, BugDorm I, BioQuip Products) at a temperature of 27–28 °C with 70–80% relative humidity on a 12:12 h L:D photoperiod. The *M. cribraria* egg masses were examined daily for 21 days for evidence of parasitism. Mainali et al. [24] in their study held *Riptortus pedestris* (F.) (Hemiptera: Alydidae) eggs parasitized by *Ooencyrtus* at 26.6 °C, 75% relative humidity, and a 16:8 h L:D photoperiod for 16 days before emergence and servers as a reference point for the duration of time *M. cribraria* parasitized eggs were observed for in this study. Adult *P. saccharalis* that emerged or failed to emerge were counted and the parasitism level was expressed as the percentage of eggs parasitized relative to the total number of eggs.

For the comparison of *Paratelenomus* and *Ooencyrtus*, a plot was selected where unusual levels of egg masses were present, but this plot was separate from all marked plots. In July and August 2017, fresh egg masses without visible evidence of parasitism were collected from this kudzu plot. Eggs were cultured as described previously.

2.3. Assessment of Parasitism in the Laboratory

Kudzu vines with roots attached, but without kudzu bugs or their eggs, were collected from the field and grown in the laboratory in quart mason jars using a solution of 30 mL of liquid fertilizer (Alaska Pure Kelp Plant Food, 0.13-0-0.06 concentrate) and 3.8 L of water. Two mason jars were placed in each of four rearing cages. Adult *M. cribraria* were collected from the field and transported to the CBC laboratory. In the laboratory, the adults were transferred into larger rearing cages (BugDorm II) containing the live kudzu vines. The vines were examined daily for the presence of kudzu bug egg masses. Newly laid *M. cribraria* egg masses were collected, starting one day after establishment. After counting the freshly laid *M. cribraria* eggs in each egg mass, the eggs were placed in glass vials (1.7 cm × 6 cm) with *P. saccharalis* adults at a ratio of two females to one male. The glass vials were then plugged with absorbent cotton moist with honey solution (1-part honey to 2-parts of tap water) to provide nourishment for the wasps. The glass vials were observed for parasitism up to 21 days. The parasitism level was expressed as the percentage of adult *P. saccharalis* that emerged relative to the number of eggs. Parasitized eggs that did not hatch were also counted.

In order to continue to culture *P. saccharalis* in winter where no eggs were available, we experimented with storing eggs of *M. cribraria* at low temperatures [24]. Some freshly laid *M. cribraria* egg masses collected from the field in spring 2016 were taken to the CBC laboratory and stored in a fridge (temperature of 2 °C) for five months [24]. Those eggs were thawed and exposed to adult *P. saccharalis* in the laboratory following the same method used for the newly laid *M. cribraria* egg masses. Parasitism level was assessed up to 21 days.

2.4. Statistical Analysis

The percentage of *M. cribraria* eggs parasitized by *P. saccharalis* was subjected to an ANOVA PROC Mixed statistical test [25]. Treatments were modelled as fixed effects; site-by-parasitoid interactions were modeled as random effects. A Tukey's studentized range test [25] was used to compare the means of parasitism between treatment groups. The data were arcsine transformed to satisfy the assumption of normality before analysis. The means of the percentage of *M. cribraria* eggs parasitized in each treatment were used to compare successful emergence of parasites and unhatched eggs using Tukey's studentized range test [25]. A significance level of 0.05 was used for all statistical tests.

3. Results

3.1. Preliminary Evaluation of Distribution on P. saccharalis in North Florida and South Georgia

In both 2016 and 2017, *P. saccharalis* emerged from eggs of *M. cribraria* collected in Leon and Gadsden County (Table 1). In Leon County for the year 2016, the parasitoid was reared from eggs in the urban area, whereas in 2017 it was seen from eggs gathered from both agricultural and urban areas. In Gadsden County, the agricultural and forested areas were monitored in 2017 and both *P. saccharalis* and *Ooencyrtus* sp. Were reared from *M. cribraria* eggs; in the forested area, *P. saccharalis* was present in both years. Host plants in agricultural sites were soybeans or kudzu growing near soybeans. All hosts in urban and forest sites were kudzu, except for the occasional presence of catnip (*Nepeta cataria* L. (Lamiales: Lamiaceae), which also hosted kudzu bugs and their egg masses, and some of these were parasitized by *P. saccharalis.*

Table 1. Average number of eggs of *M. cribraria* and presence of *P. saccharalis* (*P. s.*) in urban, forested, and agricultural areas in Leon, Gadsden, Jefferson, and Grady Counties.

Counties	2016 *M. cribraria* Eggs Sites	2016 *M. cribraria* Eggs mean ± SE	*P. s.*	2017 *M. cribraria* Eggs Sites	2017 *M. cribraria* Eggs mean ± SE	*P. s.*
Urban areas						
Leon	4	401.2 ± 57.2	Yes	3	252.6 ± 38.8	Yes
Grady	1	365.0 ± 47.5	No	1	137.4 ± 12.1	No
Forest areas						
Gadsden	1	226.2 ± 22.4	Yes	1	126.2 ± 16.8	Yes
Grady	2	83.6 ± 24.0	No	2	107.6 ± 9.8	No
Agriculture areas						
Leon	1	79.4 ± 23.1	No	1	78.4 ± 2.2	Yes
Gadsden	0	-		1	207.6 ± 21.7	Yes
Jefferson	1	36.2 ± 13.7	No	1	89.0 ± 33.4	No
Grady	1	38.2 ± 13.5	No	1	82.6 ± 19.0	No

3.2. Assessment of Parasitism in the Field

For all 12 sites studied, a total of 3804 parasitized eggs were recovered from 21,056 eggs collected (15.2% in 2016 and 16.9% in 2017). *Paratelenomus saccharalis* was found at only six sites in two counties. No parasitoids were found in Jefferson County, Florida, or Grady County, Georgia (Table 1). For sites with *P. saccharalis*, the average parasitism in 2016 varied from approximately 7% to 37% with the lowest level (7.4%) seen in the forested site in Gadsden County and the highest level (37.4%) in an urban site in Leon County (Table 2). The forested site was south of Quincy in Gadsden County; population dynamics there recorded two peaks of *M. cribraria* egg deposition (Figure 2: 204 eggs in May and 281 eggs in July). Similarly, the most active urban site in Leon County also had two peaks of egg deposition (Figure 3: 504 eggs in May and 428 eggs in August). In contrast, the most active agricultural site located at the USDA-ARS soybean research field (Leon County) recorded a single peak of *M. cribraria* egg deposition (Figure 4: 130 eggs in August). For 2016 and 2017 study period, it was observed on the graphs (Figures 2–7) that there were two peaks in parasitism in urban and forested areas where kudzu vine was the host crop and a single peak in agricultural area where soybean was the host crop. The first peak of parasitism in the nonagricultural areas coincide with the vigorous growth of kudzu vines right after the winter.

Table 2. Within-area comparison of *M. cribraria* eggs parasitized by *P saccharalis* in 2016 and 2017. Parasitism expressed as a percentage. Within each column, means followed by common letters are not significantly different (Tukey's Studentized Range; $p < 0.05$).

Areas	Sites	% Parasitism (Mean ± SE) 2016	% Parasitism (Mean ± SE) 2017
6a. Urban			
Leon	1	23.40 ± 3.40 a	- [a]
Leon	2	20.00 ± 7.49 a	8.80 ± 2.60 b
Leon	3	00.00 ± 0.00 c	00.00 ± 0.00 c
Leon	4	37.40 ± 4.16 a	29.8 ± 3.72 a
Grady	5	00.00 ± 0.00 c	00.00 ± 0.00 c
6b. Forested			
Gadsden	6	7.40 ± 3.94 a	35.00 ± 3.94 a
Grady	7	00.00 ± 0.00 c	00.00 ± 0.00 c
Grady	8	00.00 ± 0.00 c	00.00 ± 0.00 c
6c. Agricultural			
Leon	9	00.00 ± 0.00 c	61.40 ± 5.01 a
Gadsden	10	- [b]	25.20 ± 4.50 b
Jefferson	11	00.00 ± 0.00 c	00.00 ± 0.00 c
Grady	12	00.00 ± 0.00 c	00.00 ± 0.00 c

[a] Site 1 kudzu patch was cleared in early 2017. [b] Site 10 not collected in 2016.

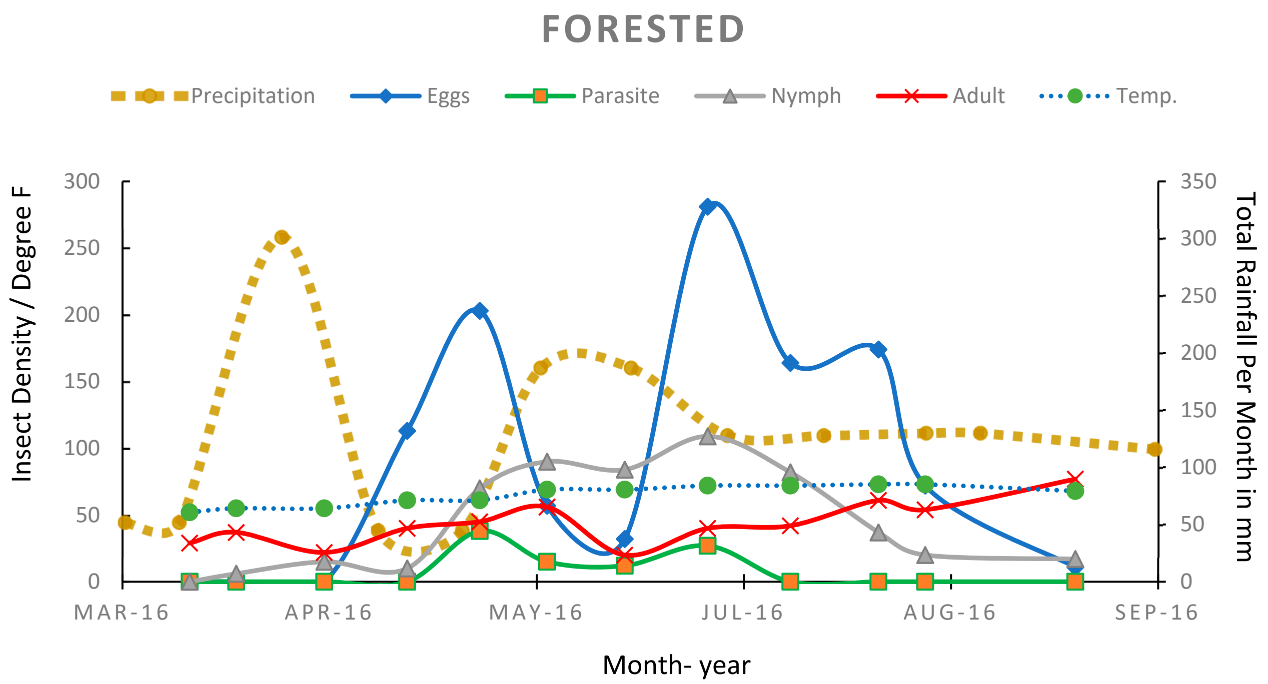

Figure 2. Population dynamics of *M. cribraria* and *P. saccharalis* for most active forested site in 2016. Host plant: kudzu.

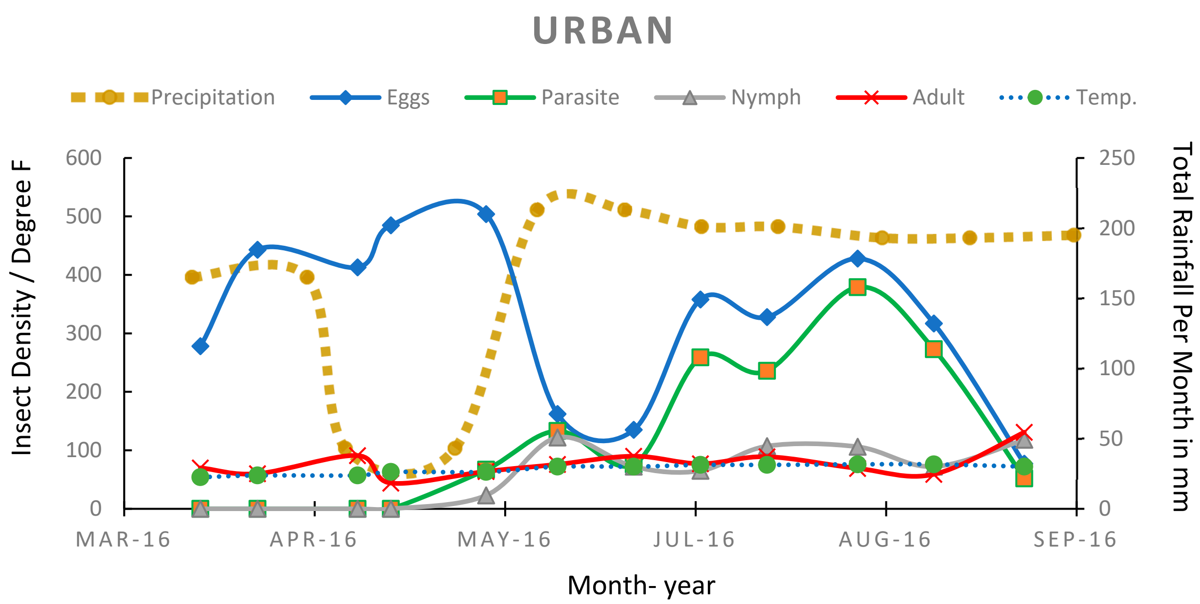

Figure 3. Population dynamics of *M. cribraria* and *P. saccharalis* for most active urban site in 2016. Host plant: kudzu.

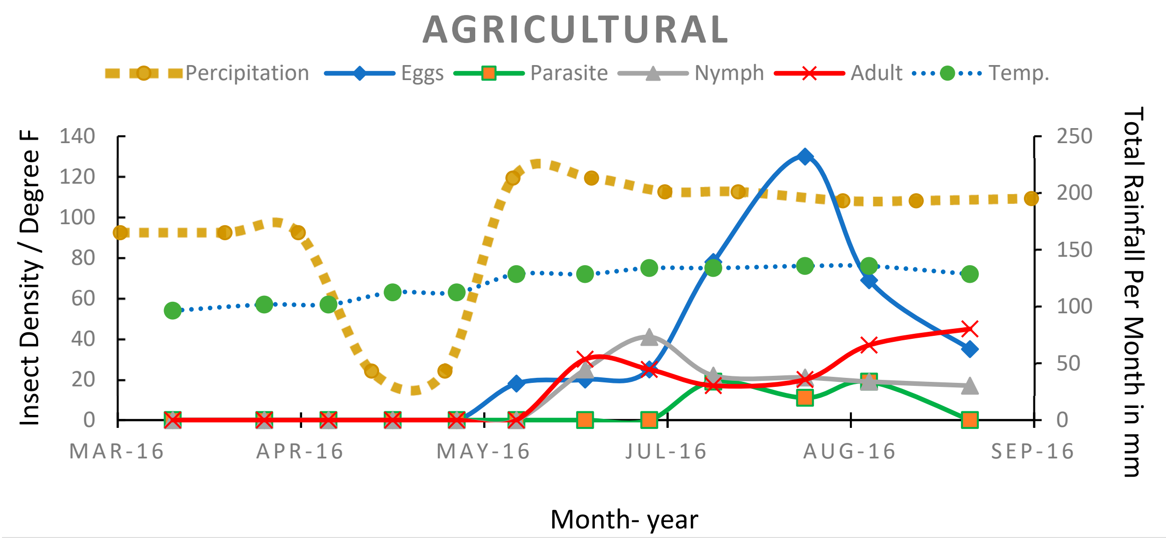

Figure 4. Population dynamics of *M. cribraria* and *P. saccharalis* for most active agricultural site in 2016. Host plant: soybean.

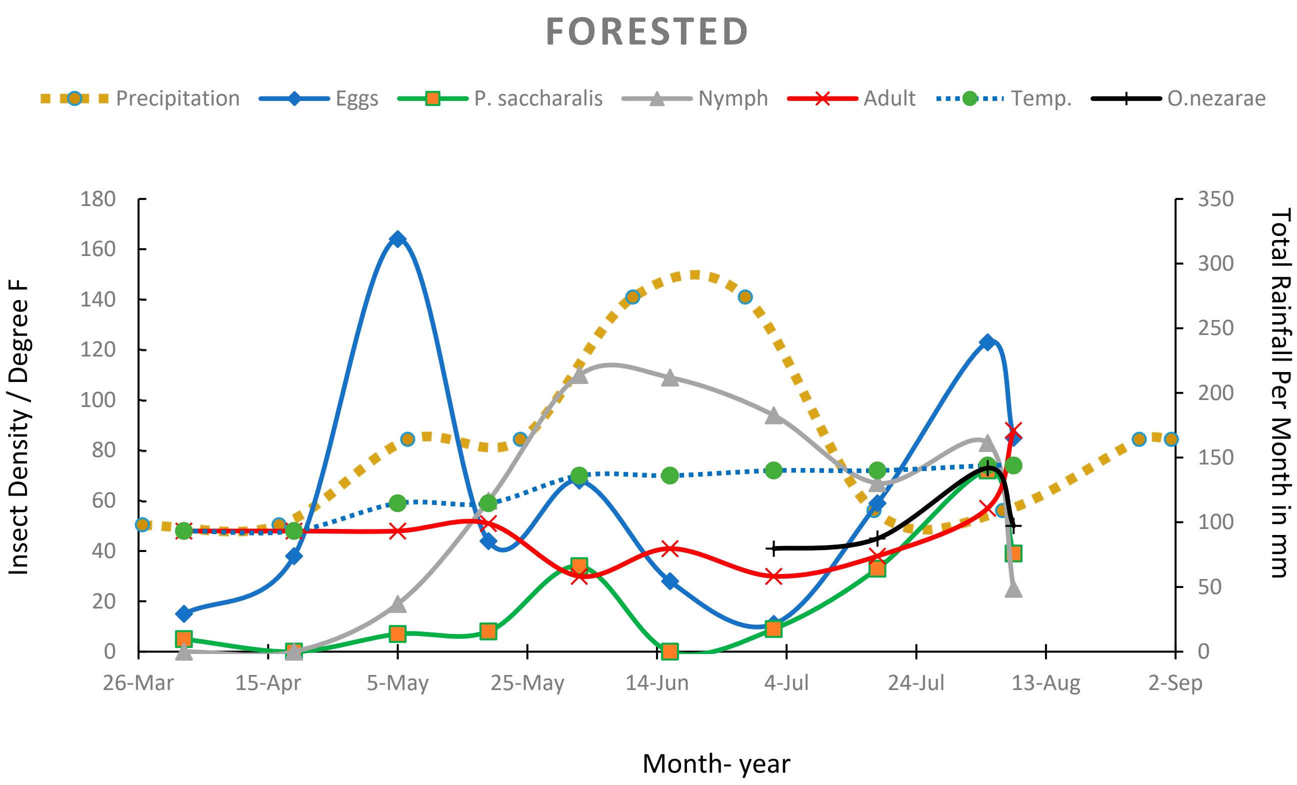

Figure 5. Population dynamics of *M. cribraria* and *P. saccharalis* for most active forested site in 2017. Host plants: *Pueraria montana* (kudzu).

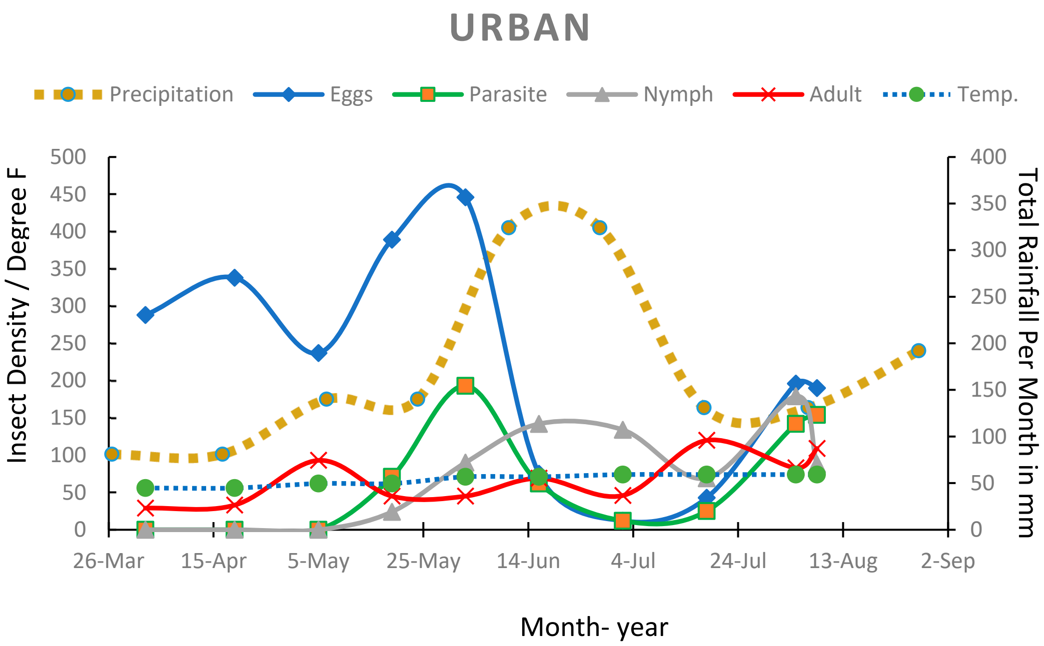

Figure 6. Population dynamics of *M. cribraria* and *P. saccharalis* for most active urban site in 2017. Host plant: *Pueraria montana* (kudzu).

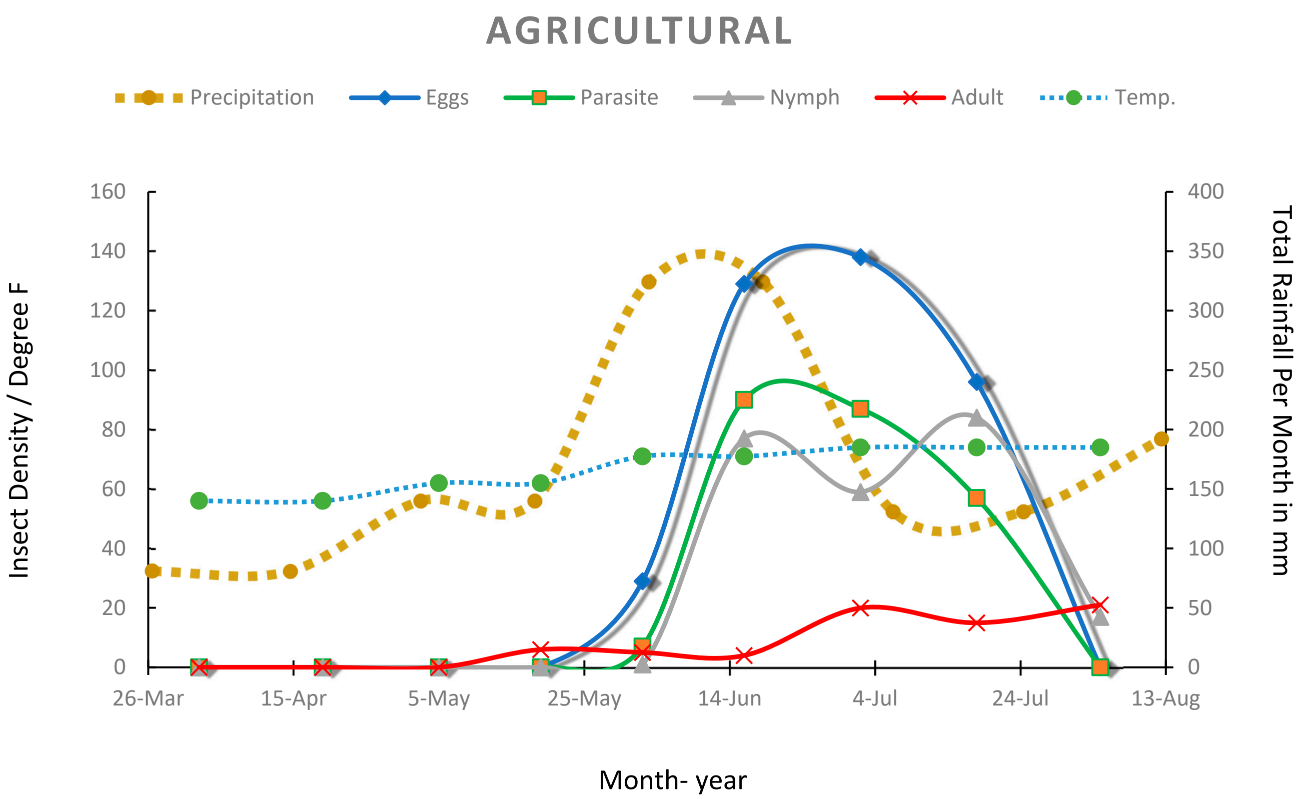

Figure 7. Population dynamics of *M. cribraria* and *P. saccharalis* for most active agricultural site 2017. Host plant: soybean.

Differences were observed in the average level of parasitism within the urban and forest area in both years, and in the agricultural area for 2017 (Table 2). In 2017, the lowest level of parasitism (8.8%) was recorded at another urban site in Leon County, whereas the highest level (61.4%) was found at the USDA-ARS agricultural research site in Leon County

(Table 2). As in 2016, population dynamics in 2017 for the most active urban and forested sites (Figures 5 and 7) showed two peaks of *M. cribraria* egg deposition: 338 eggs in April and 446 eggs in June, and 164 eggs in May and 123 eggs in August, respectively. Population dynamics for the USDA-ARS agricultural site in Leon County showed a unimodal pattern similar to that of 2016 (Figure 6: one peak of 138 eggs in July).

Between-group comparisons indicated significant differences ($F = 13.77$; $df = 2, 52$; $p < 0.0001$) between the average parasitism levels in agricultural, urban, and forested areas in 2016; in 2017, the differences between areas were not significant ($F = 1.96$; $df = 2, 52$; $p = 0.1566$). The total level of parasitism for all sites increased from 8.02% in 2016 to 14.56% in 2017 (Figure 8), and the between-year difference was significant ($F = 3.80$; $df = 1108$; $p = 0.0537$).

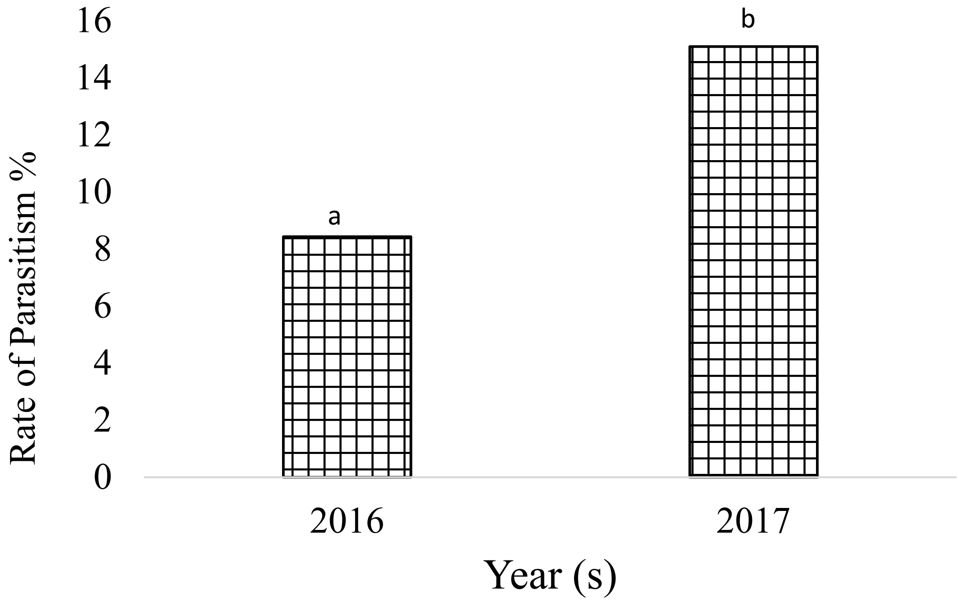

Figure 8. Total egg parasitism by *P. saccharalis* on *M. cribraria* in the three areas (urban, forest, and agricultural) compared by year. Different letters above the bar indicates significant difference between the years, Tukey's Studentized Range, $p < 0.05$).

Results summarized above (Table 3, Figure 8) compare the four-county study region. Levels of parasitism may also be expressed for only the sites where *P. saccharalis* was present. Parasitism at these sites over two years averaged 29.1%. Considering only parasitized egg masses, the rate of parasitism varied from 27.8–100% with an average of 70% for both years (71% in 2016, 69% in 2017). Data from the preliminary study of dual parasitism by *P. saccharalis* and *O. nezarae* are summarized in Table 4. These results are from a plot adjacent to the field plots. All egg masses were parasitized, and many eggs were parasitized by both species.

Table 3. Between area comparison of *M. cribraria* egg parasitized by *P saccharalis* in 2016 and 2017. Within each column, means followed by common letters are not significantly different (Tukey's Studentized Range; $p < 0.05$).

Areas	**Percentage Parasitism (Mean ± SE)**			
	Sites	**2016**	**Sites**	**2017**
Urban	5	16.16 ± 3.39 a	4	9.65 ± 2.98 b
Forest	3	2.47 ± 1.54 c	3	11.67 ± 4.59 b
Agriculture	3	0 ± 0 c	4	21.65 ± 5.97 b

No.: represent number of replications per area.

Table 4. Comparison of parasitism of egg masses and eggs from sites with both *P. saccharalis (P.s.)* and *Ooencyrtus nezarae (O.n.)* in 2017.

.	Egg Masses of *M. cribraria*				Eggs of *M. cribraria*			Ratio *P.s./O.n.*
		Parasitized by				Parasitized by		
Date	**No.**	***P.s.***	***O.n.***	**Both**	**No.**	***P.s.***	***O.n.***	
3 July	2	0	0	2	33	13	15	1:1
18 July	3	1	1	1	54	5	26	1:5
8 August	3	2	0	1	47	24	8	3:1
13 August	7	3	2	3	162	69	23	3:1
Total	15	6	3	7	296	111	72	8:8
Parasitized		40%	13%	47%		37%	24%	

In 2016, precipitation was broadly bimodal throughout all habitats, with a first peak in March and April followed by a decline in May and a second prolonged increase from July to November. In 2017, a single peak occurred in May to July in all habitats. Precipitation was focused on more than temperature in this population dynamics study since the result of increased precipitation over time can influence vigorous growth in host plants. New growth in host plants encourages kudzu bug egg deposition. Conversely, the impact of precipitation on the kudzu bug population can be negative. Precipitation can wash away nymphs, and increased moisture in the field encourages the growth of entomopathogens which will result in population reduction. Average minimum temperature did not differ much throughout the season (Figures 2–7).

3.3. Assessment of Parasitism in the Laboratory

In laboratory experiments, 911 freshly laid eggs were compared with 349 *M. cribraria* eggs, which had been cooled for five months (Table 5). They were isolated and offered to predators to determine the level of parasitism in the absence of environmental factors and competing predators. After 21 days of observation, an average of 77.4% of freshly laid and 61.6% of cooled eggs were parasitized by *P. saccharalis* and an adult wasp emerged. There was a significant difference ($F = 5.16$; $df = 1, 60$; $p > 0.0268$) in emergence of freshly laid eggs over cooled eggs (Table 5).

Table 5. Comparison of emergence of *P. saccharalis* (*P.s.*) from freshly laid eggs and cooled eggs of *M. cribraria* in laboratory experiments.

Experiment.	No. Egg Masses	No. Eggs	Eggs per Mass	*P.s.* Emerged	
			Mean + SE	No.	Mean ± SE
Fresh eggs	45	911	20.2 ± 1.0	705	0.81 ± 0.03 a
Cooled eggs	17	349	20.5 ± 1.2	215	0.67 ± 0.07 b

a and b means there was a significance difference between the two groups.

4. Discussion

The presence of the wasp *P. saccharalis* was confirmed in north Florida and, unexpectedly, *Ooencyrtus* sp. was also discovered. This is the first record from Florida, but *Ooencyrtus* sp. Has been found in Virginia in 2015 [26]. From March to September of 2016 and March to August of 2017, *P. saccharalis* was reared from eggs of *M. cribraria* in two of four counties studied and in three environmental areas: urban, forest, and agricultural. The habitat diversity of this wasp parasitizing kudzu bug eggs has been documented in many parts of the world, including the United States [26–28].

Hoshino et al. [27] investigated seasonal occurrence of the wasp in Japan in urban areas of Fukuoka and Tokyo where *P. saccharalis* coexists on kudzu bug eggs with the generalist parasitoid *Ooencyrtus nezarae*. Based on research in Japan [27], the two parasitoids can occur on the same host together, however, some seasonal separation was observed, as *P. saccharalis* was found earlier in the season. When both species occurred together, the only measurable effect of this coexistence was an increase in the relative number of male *O. nezarae*, but more studies on their interactions are needed [27]. Nothing is known concerning the importance or interactions of the two parasitoids in Florida.

This appears to be the first record of the adult kudzu bug on catnip; however, this study did not follow growth on catnip to determine if the kudzu bug could complete its life cycle on this host, and only eggs and first instar nymphs were observed. *Paratelenomus saccharalis* did parasitize those available kudzu bug eggs deposited on catnip. Catnip may be an incidental record, as we observed that the kudzu bug seems to deposit its eggs on any available surface. Egg masses were observed on dry twigs and catnip in the field and on the sides of rearing cages in the laboratory.

In the field evaluation of *M. cribraria* egg masses, significant differences were found in *M. cribraria* egg parasitism by *P. saccharalis* within sites of collection for both years (Table 2). Parasitism was highest within urban sites in 2016 and this could be a result of favorable weather conditions for the kudzu plants in early spring. This contributes to robust growth for the host plant, and healthy plants attract *M. cribraria*. In 2017, one of the urban sites was destroyed, and the other sites had a reduced level of parasitism in that year. Agricultural sites for 2017 recorded levels of parasitism up to 61.40% from no parasitism in 2016.

Variability in the level of parasitism was also seen in many studies in different environments where the kudzu vine is prevalent. High levels of parasitism were seen on kudzu in Japan where the average parasitism for *M. cribraria* eggs by *P. saccharalis* varied from 57–81% [29]. In Georgia, Gardner et al. [17] reported 47.5% parasitism on kudzu bug egg masses collected in DeKalb County. On another leguminous host, Bin and Colazza [30] recorded an average range of parasitism by *P. saccharalis* of 4.5–34.2%.

There was also a significant difference in the average level of parasitism between the agricultural, urban, and forested areas in 2016, although there was no difference in the mean rate of parasitism between these areas in 2017 (Table 3). Parasitism increased in the agricultural areas from 0% in 2016 to 21.7% (±6.0%) in 2017. Forest areas also experienced an increase from 2.5% (±1.5%) to 11.7% (±4.6%) in 2016 and 2017, respectively. Zhang et al. [5] reported that the number of *M. cribraria* egg masses in different seasons may also affect the rate of parasitism. The single peak in soybean parasitism may have been related to its late May planting season. Kudzu vines would be available in mid-April while soybeans would not be available for invasion until June, which would coincide with the second peak of egg population on kudzu. The fungus *Beauveria bassiana* was also observed at the agricultural site (USDA-ARS) infecting and killing all stages of kudzu bugs in 2016 (Diedrick, unpublished data).

In July and August of 2017, *Ooencyrtus nezarae* were reared with *P. saccharalis* from *M. cribraria* egg masses. The parasitism of kudzu bug egg masses by these two egg parasitoid species has been reported in many studies where the two were found competing with each other [8,26]. Although 100% of egg masses contained parasites, only 61% of eggs were actually parasitized (Table 4). The ratio of *Ooencyrtus* was higher in July than in August which differs from results in Japan [27]. The work by Ademokoya et al. [16] did not compare populations, but it seems clear that the Alabama and Florida populations are not as established as those in Asia, and it will be interesting to follow the coexistence of the two parasitoids. While *Ooencyrtus* parasitizing kudzu bugs represents one more possible biological control agent, it is a generalist predator, and more research is needed to evaluate its importance in Florida.

In order to maintain a population of *P. saccharalis* over winter, we tried to see if cultures of *M. cribraria* eggs could be kept long term by freezing eggs in the summer and thawing them later. A second evaluation of parasitism of kudzu bug eggs under controlled conditions, using both fresh and cooled eggs, revealed a significantly poorer emergence rate of *P. saccharalis* on cooled eggs compared to fresh eggs (Table 5). However, numbers were sufficient to maintain the culture during periods when freshly laid *M. cribraria* eggs were not available.

The egg parasitoid *Paratelenomus saccharalis* is established in the state of Florida. This parasitoid of *Megacopta cribraria* was confirmed in Leon and Gadsden counties after previously being found in Alachua County, where it is no longer present; however, it was not found in adjacent areas in Jefferson County. In 2016, *P. saccharalis* was found in three urban sites in Leon County (Mahan Dr., Eugenia St., and Tom Brown Park) and one forested site in Gadsden County (south of Quincy); in 2017, its distribution also included two sites in agricultural areas (USDA-ARS in Leon County and UF-NFREC in Gadsden County).

The kudzu bug was first found in Georgia in 2009 and reached Florida three years later. *Paratelenomus saccharalis* was first recorded in Georgia, Alabama, and Mississippi in 2013 [31], and in 2014 in Florida [28]. *Paratelenomus saccharalis* has not been found in Georgia since 2015, and it was not found at sites in Grady County, Georgia, in this survey. However, the kudzu bug population significantly declined in Georgia [31] between the years 2013 and 2016. The decline was initially attributed to *P. saccharalis* and later to the fungus *Beauveria bassiana* [31]. Populations of *P. saccharalis* also declined, presumably because of unusually cold winters [31].

In this study, the levels of parasitism of egg masses of the kudzu bug by the parasitoids varied across sites, areas, and counties, and over the study period. *Paratelenomus saccharalis* is specific to the family Plataspidae and thus host-specific to *M. cribraria*. In 2017, we also found a second egg parasitoid of the kudzu bug, *Ooencyrtus nezarae*. The survival of this recently discovered generalist parasitoid may be affected by high and low temperatures [32], but the ability of *Ooencyrtus* to find alternate hosts may make it easier to maintain a population of natural biocontrol agents against kudzu bugs in the future. In addition to Georgia, seven other states have closely followed the population of the kudzu bug on soybean since 2011 [33,34]. The population has declined in states where it was initially found (North Carolina, then Alabama) but populations are increasing in Mississippi, Tennessee, and now in two of three other states (Arkansas and Louisiana). Although reported in Virginia, it has not yet affected the soybean crop [34,35] where it is being closely monitored.

Agricultural regions carefully monitor invasive species and apply insecticides when needed following extension recommendations, so it appears that the integrated pest management approach is slowly reducing kudzu bug populations in soybean fields. This decline is also due at least in part to natural enemies (parasitoids and fungi). Control of kudzu bugs in urban areas is dependent on the management of kudzu by individual property managers; the heaviest kudzu bug infestations in this study were not on well-kept property. Both the total number of kudzu bug eggs and the parasitoid population density declined over the two years of the study, but not at all sites. Geographically, the population may be in decline in parallel with Florida's close neighbors Georgia and Alabama. However, our study was limited to only four counties, mainly in northern Florida and southern Georgia. Therefore, more research is needed to confirm any decline; to continue exploring the characteristics and behavior of the parasitoid in other counties, and eventually in other states where the parasitoid is prevalent.

5. Conclusions

This study suggests that both *P. saccharalis* and *O. nezarae* exert some levels of natural control of the kudzu bug in the field and may be useful as biological control agents in an integrated pest management program. This new knowledge on the parasitism level and population dynamics of the kudzu bug in north Florida can enhance management programs to better manage this invasive pest while protecting natural enemies.

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