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Green Lacewing (*Chrysoperla rufilabris*) is a Voracious Predator on Crapemyrtle Bark Scale (*Acanthococcus lagerstroemiae*)

Bin Wu[1], Runshi Xie[1], Mengmeng Gu[2*], Hongmin Qin[3]

[1]Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843, USA; [2]Department of Horticultural Sciences, Texas A&M AgriLife Extension Service, College Station, TX 77843, USA; [3]Department of Biology, Texas A&M University, College Station, TX 77843, USA.

mgu@tamu.edu ; hqin@bio.tamu.edu

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Summary

Crapemyrtle bark scale (CMBS, *Acanthococcus lagerstroemiae*), an invasive sap-sucking hemipteran, has spread across 14 states of the United States. The infestation of CMBS negatively impacted the flowering of some ornamental plants, and even the fruiting of some economically important crops. Using natural enemies, a non-chemical approach, would be beneficial for the integrated management of CMBS. Eggs of the green lacewings were observed on CMBS-infested crapemyrtle plants at Texas A&M University campus. Aiming to utilize green lacewings (*Chrysoperla rufilabris*) as a biocontrol agent of CMBS, predatory capacity of the green lacewings on CMBS was evaluated in laboratory conditions in this study. The results confirmed that the larval green lacewings could prey upon CMBS's nymphs, eggs, and adults. The average duration of the first egg consumption (P<0.0001) and the mean number of CMBS eggs consumed per larval green lacewing in 24 hours (P<0.0001) differed among different developmental stages. The 1st instar lacewing took 141.4 ± 4.8 sec. (mean ± SE) to consume the first CMBS egg and finished 11.8 ± 1.3 CMBS eggs in 24 hours. Whereas, the 3rd instar green lacewings devoured the first egg in 60.3 ± 3.0 seconds and consumed 176.4 ±

6.9 eggs per 24 hours. The Y-tube assay demonstrated that 78.1 ± 4.7% of larval *C. rufilabris* located CMBS under dark conditions. Thus, the evaluation of the predation capacity and Y-tube results confirmed that *C. rufilabris* could potentially be applied to control CMBS biologically.

INTRODUCTION

Crapemyrtle bark scale (CMBS, *Acanthococcus lagerstroemiae*) is a sap-sucking pest introduced from other countries (Gu et al., 2014; Merchant et al., 2014). Similar to aphids, crapemyrtle bark scale secretes honeydew when feeding on a plant. The infestation of CMBS seriously affects host plants' growth and development, even leading to branch die-back (Wang et al., 2019). This exotic pest was firstly reported in Texas in 2004 and has rapidly spread to 14 states, including Alabama, Arkansas, Georgia, Kansas, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia (EDDMapS, 2020), and Washington (personal communication).

Crapemyrtle bark scale is a polyphagous insect and threatening many plants. In our previous study, different *Lagerstroemia* species and *Callicarpa* species (*L. indica* 'Dynamite', *L. fauriei* 'Kiowa', *L. limii*, *L. subcostata*, *L. speciosa*, *C. dichotoma* 'Issai', *C. americana* 'Bok Tower', *C. longissima* 'Alba', and *C. randaiensis*) were confirmed as CMBS hosts (Wu et al., 2019). The CMBS infestation was also observed on apple (*Malus domestica*), twelve pomegranate cultivars (*Punica granatum*), and other crop plants (Xie et al., 2020).

Natural enemies could be utilized to control CMBS biologically. In the field and laboratory conditions, cactus lady beetles (*Chilocorus cacti*) were confirmed as a predator on CMBS in Louisiana and Texas (Wang et al., 2016a; Wang et al., 2016b). We noticed that eggs of green lacewings (*Chrysoperla rufilabris*) were found feeding on CMBS-infested crapemyrtle plants at Texas A&M University campus (Fig. 1).

Figure 1. Several larval *Chrysoperla rufilabris* were observed feeding on CMBS female adults and lacewing eggs were found on CMBS-infested plants under natural environment.

Green lacewing is a highly fecund holometabolous insect, averaging 284 eggs per oviposition (Albuquerque et al., 1994). *Chrysoperla carnea* and *C. rufilabris* have been applied for pest biocontrol for many years in greenhouse and field crops (Tauber et al., 2000). The predation capacity of *C. rufilabris* on CMBS has not been reported yet.

Volatile communication is vital in moderating the interactions between insects, host plants, and other organisms (de Vos and Jander, 2010; Sudhida et al., 2010; Van Emden and Hagen, 1976; Zhu et al., 2005). Electroantennography (EAG) response was recorded from *C. carnea* in response to semiochemicals released from the prey (Zhu et al., 1999). Similarly, *Chrysoperla rufilbaris* searching CMBS may involve olfactory. Thus, foraging behavior and predatory capacity upon CMBS eggs in 24 hours was investigated under laboratory conditions to confirm *C. rufilabris* as an effective biocontrol agent of CMBS. A Y-tube assay was conducted to test if the lacewings could locate CMBS under dark.

MATERIALS AND METHODS

Insects. Larvae of *C. rufilabris* were purchased from ARBICO Organics™ (Oro Valley, AZ) and reared individually in Petri dishes (5.5 cm diameter) in a CONVIRON® -BDR 16 growth chamber (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) at 25 ± 1℃ and $60 \pm 5\%$ relative humidity (R.H.) under a 12:12 (light: dark) photoperiod. An artificial diet containing 500mM sucrose, vitamins, minerals, and 150 mM amino acids (Prosser and Douglas, 1992) was used as food for the green lacewings. Larval *C. rufilabris* was held individually in a Petri dish (5.5 cm in diameter) and starved for 4hr before utilization in the tests. Additionally, the larvae were starved for 24 hours before their use in the June test.

Nymphs, adults, and eggs of CMBS were collected from naturally CMBS-infested crapemyrtle plants in College Station, TX.

Experiment 1. Can green lacewings prey on CMBS in lab conditions?

Crapemyrtle bark scale nymphs, adults, and eggs were distributed in a Petri dish (5.5 cm in diameter) to test whether *C. rufilabris* larvae would effectively prey on CMBS. Then, a larval *C. rufilabris* was placed in the same Petri dish. The preying behavior of the green lacewing was investigated using Stemi 2000 stereomicroscope (Carl Zeiss AG, Oberkochen, Germany) in laboratory conditions.

Experiment 2. How many CMBS eggs does a larval green lacewing consume in 24 hours?

The predatory evaluation experiment was conducted, respectively, in June and October 2019. In the June test, an individual 24h starved green lacewing was introduced into the center of a Petri dish (5.5 cm in diameter) containing approximately 300 fresh CMBS eggs. The duration when a larval *C. rufilabris* consumed the first CMBS egg entirely was recorded. After 24 hours, the number of CMBS eggs consumed by *C. rufilabris* was counted with the help of ImageJ (a Java-based image processing program developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation). An image of the eggs in the Petri dish before feeding was taken to easier compare and confirm the number of eggs consumed by the larval green lacewing in the 24 hours. The 24hr predation test was repeated six times, tracking the same individual *C. rufilabris* with a 24hr starvation as an interval. Thirteen effective repetitions were recorded and plotted using Excel.

In the October test, an individual 1st, 2nd, or 3rd instar larva of the starved green lacewing was inoculated into the center of a Petri dish (5.5 cm in diameter) containing approximately 300 fresh eggs of CMBS. The duration when lava consumed the first CMBS egg completely was recorded. After 24 hours, the number of CMBS eggs consumed by *C. rufilabris* was counted with the help of ImageJ. A set of twenty dishes was replicated three times for each instar. For the data analysis, the duration of the first egg consumption and the prey consumption of the green lacewing were analyzed using repeated-measures ANOVA.

Stages, replicates, the stages*replicates interaction were the variables, assigned subjects nested with stages as a random effect. The means of the duration and consumption per 24hr were, respectively, separated using Tukey's HSD test at α =0.05 (JMP Pro 15, SAS Institute, Cary, NC).

Experiment 3. Are green lacewings able to forage CMBS in the dark?

A Y-tube assay was set up in this study (Fig. 2). Three glass vials were joined by a Bel-Art Y-tubing connector (SP Scienceware, Wayne, NJ). The loading vial contained a starved second or third instar green lacewing, the baited vial contained over ten alive gravid females and some crawlers, and the control vial was vacant. The Y-tube setting was boxed to avoid visual stimuli, and the box was put into the CONVIRON® -BDR 16 growth chamber at 25±1℃ and 60±5% R.H. under a 24-hour dark photoperiod.

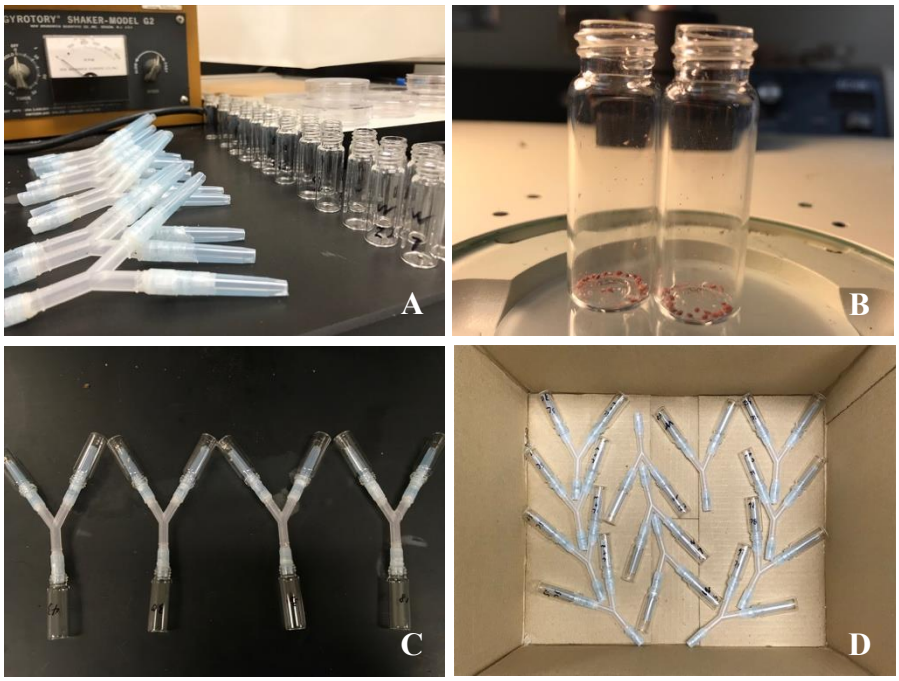

Figure 2. Y-tube test assemble**.** A: Y-tube tubes and vials; B: Vials containing CMBS; C: Each Y-tube set up was assembled by a Y tubing connector (6.0 mm tubing I.D.) and contained a loading vial, a baited vial and a control vial; D: Y-tube tests were conducted under dark.

Twelve Y-tube settings were performed simultaneously. After 24 hours, the number of predators that entered the baited vials (B) and the control vials (C) was counted. The positive response ratio (%) was calculated as B/(B+C) *100, and the negative response ratio (%) was calculated as C/(B+C) *100. The test was repeated ten times using fresh CMBS and larval green lacewings. The data were analyzed using one-way ANOVA (JMP Pro 15, SAS Institute, Cary, NC), and the response ratios were separated using Tukey-Kramer HSD (α = 0.05).

RESULTS AND DISCUSSION

Prey trial of larval *C. rufilbaris* on CMBS in laboratory conditions. The larval green lacewing was observed to devour gravid females and eggs of CMBS in the Petri dish (Fig. 3 and 4). Moreover, it was able to grab and devour tiny crawling nymphs under the lab conditions (Fig. 4). Together with observation in landscapes, green lacewings are probably natural biological control agents of CMBS.

Figure 3. Nymphs of *Chrysoperla rufilabris* were preying on female adults of CMBS under laboratory condition**.** A: The larval green lacewing easily grasped and voraciously attacked a female adult of CMBS by seizing it with its large, sucking jaws after placing them together in the same petri dish; B: The larva of *C. rufilabris* esuriently consumed the body fluids of the CMBS female leading to the CMBS shrinking and death.

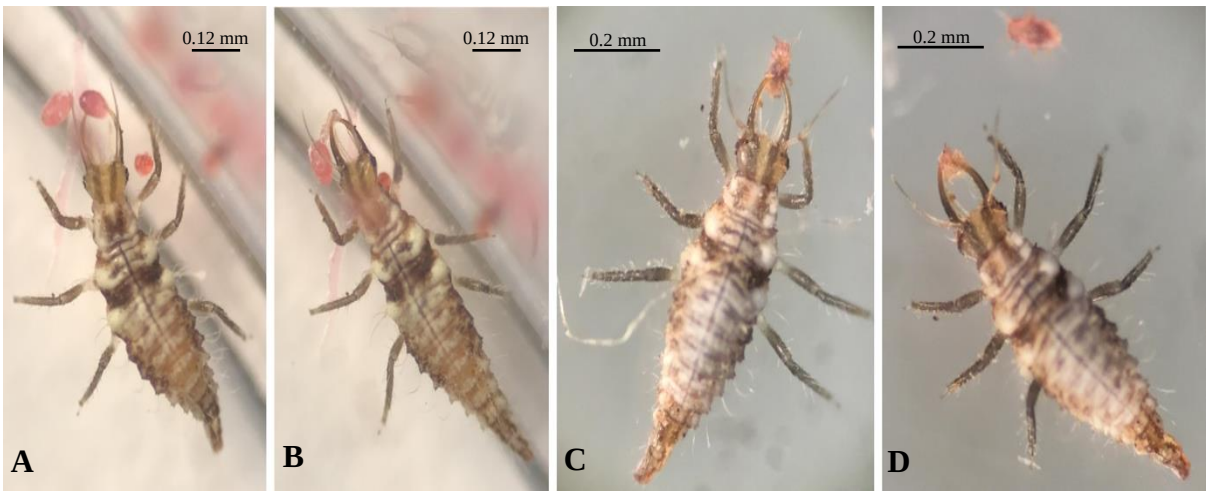

Figure 4. Larval green lacewings were able to prey on eggs and crawlers of CMBS under laboratory conditions**.** A: The larval *C. rufilabris* grasped a CMBS egg; B: The larva consumed the egg in around one minute after grabbed it; C: A larva of *C. rufilabris* seized a CMBS crawler; D: The larva rapaciously pierced the crawler.

Evaluation of the predatory capacity upon CMBS eggs. In the June test (Fig. 5), the duration of the first egg consumption ranged from 53.2 ± 2.5 seconds to 73.2 ± 2.7 seconds. The average number of CMBS eggs that the larval green lacewing consumed ranged from 154.1 ± 2.7 to 195.5 ± 2.5. In the October test, the analysis result of fixed effect tests showed that only the developmental stages of *C. rufilabris* impacted the duration of the first egg consumption (P<0.0001) and the number of CMBS eggs eaten in 24 hours (P<0.0001).

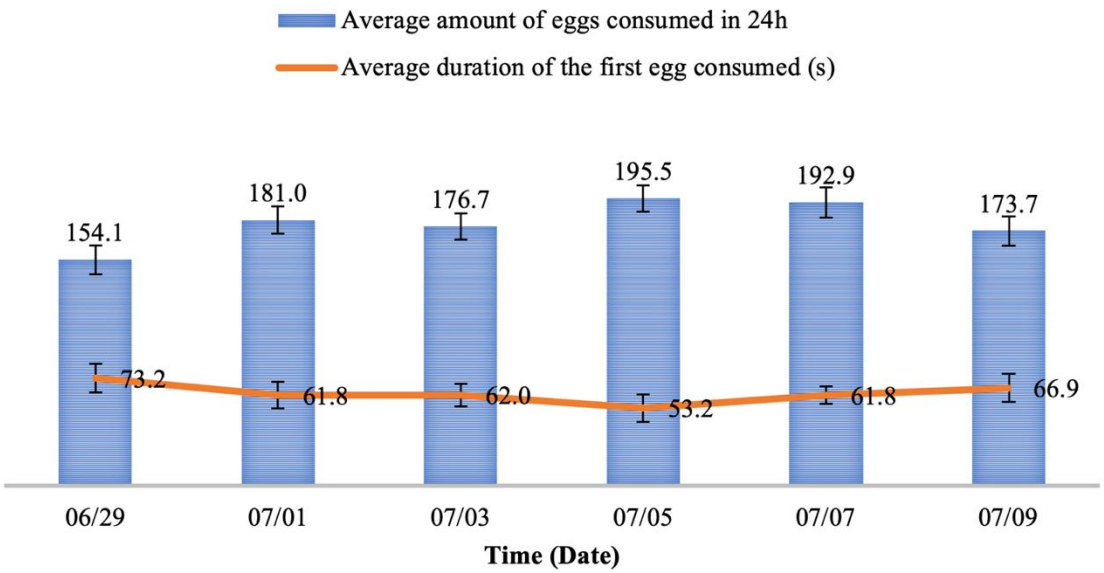

Figure 5. 12-day tracking results on the mean amount of CMBS eggs consumed by a larval *Chrysoperla rufilabris* in every 24 hours (blue columns) and the average duration of the first CMBS egg consumed (orange line).

Subsequently, the three-replicate data for each development stage, 60 dishes as a set, were calculated and shown in Table 1. The average duration of the first egg consumption dropped from 141.4 ± 4.8 seconds (mean ± SE) in the 1st instar to 60.3 ± 3.0 seconds in the 3rd instar. The average number of CMBS eggs consumed by a larval green lacewing in 24 hours increased dramatically from 11.8 ± 1.3 in the 1st instar to 176.4 ± 6.9 in the 3rd instar.

The results in Experiment 1 demonstrated that larval green lacewing voraciously preyed on crawling nymphs, adults, and eggs of CMBS. However, similar to lady beetle species (Bilde and Toft, 1997; Bilde and Toft, 1999; Finlayson et al., 2010; Golizadeh and Jafari-Behi, 2012; Omkar and Sahu, 2009), green lacewings showed significantly feeding preference among aphid species (Chen and Liu, 2001; Liu and Chen, 2001).

Table 1. The effect of developmental stages on prey consumption of *Chrysoperla rufilabris* upon CMBS eggs.

Developmental stage	Number of larval green lacewings tested	Replicate	Duration of the first egg consumption (seconds ± S.E.)	Mean number of CMBS consumed in 24 hours (± S.E.)
1st instar	20	3	141.4 ± 4.8a	11.8 ± 1.3c
2nd instar	20	3	77.5 ± 4.7b	151.5 ± 6.6b
3rd instar	20	3	60.3 ± 3.0c	176.4 ± 6.9a

* Means, in the same column, followed by different letters are significantly different (P<0.05) as determined by Tukey's HSD test.

The 3rd instar of the lacewings can consume at most 277 CMBS eggs in Experiment 3, compared with approximately 400 scale eggs eaten by the 4th instar of *C. cacti* in 24 hours under the lab conditions (Wang et al., 2016a). Thus, to select the optimal predator in effectively controlling CMBS, it would be necessary and interesting to compare the predation capacity on CMBS between some lady beetle species and the green lacewing species.

Olfactory response using a Y-tube assay. The analysis results showed that the positive response ratio was significantly higher than the negative ratio (P<0.0001), which indicated that the lacewings were attracted to CMBS in the Y-tube settings. In detail, 78.14 ± 4.74% of the larval green lacewings were able to locate CMBS in the Y-tube assay under dark (Fig. 6).

Electroantennography responses to some sex pheromone components or alarm pheromones of the aphids (Homoptera: Aphididae) have been obtained using green lacewings, *Chrysopa cognata* (Boo et al., 1998; Cho et al., 2014) and *Chrysopa pallens* (Li et al., 2017), and other natural enemies like *Coccinella septempunctata* (Al Abassi et al., 2000) and *Adalia bipunctata* (Francis et al., 2004). Some pheromone compounds were investigated and proved influential in the field trapping experiment (Boo et al., 2003; Zhang et al., 2006). Similarly, spraying the extraction of CMBS volatile compounds could be technologically helpful to enhance the efficiency of CMBS biocontrol before the infestation happens heavily on a plant. Thus, it would be beneficial to focus on confirming and extracting the specific compounds in the future study.

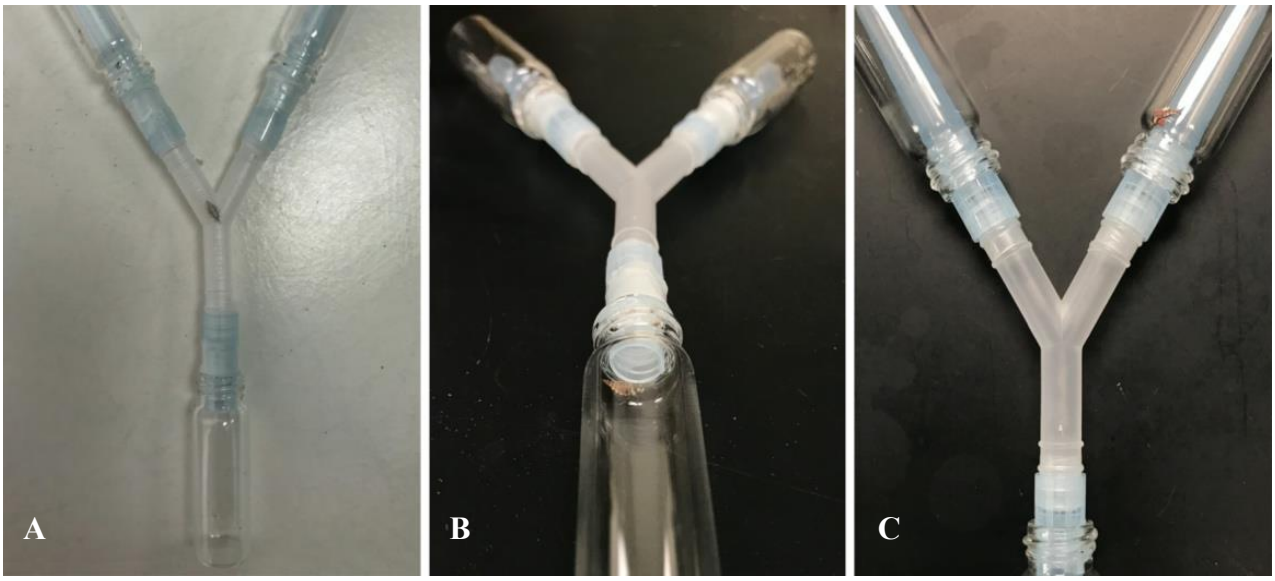

Figure 6. Different responses in the Y-tube test under dark. After 24 hours, some larval green lacewings went to the control vials (A) or stayed in the loading vials (B) Still, 78.14 $\pm$ 4.74% of the larval *C. rufilabris* were able to locate CMBS in the baited vials.

Acknowledgments

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