Host Preference and Development of *Leptoglossus zonatus* (Hemiptera: Coreidae) on Satsuma Mandarin

YINGFANG XIAO and HENRY Y. FADAMIRO

Department of Entomology and Plant Pathology, Auburn University, AL 36849

**ABSTRACT** *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) has recently emerged as a key pest of satsuma mandarin, *Citrus unshiu* Marcovitch, and other fruit crops in the Gulf Coast region of the United States. Studies were conducted under laboratory conditions (25 ± 2°C, 50 ± 10% RH, and a photoperiod of 14:10 [L:D] h) to investigate host preference and suitability of satsuma fruit as host for this pest. Three separate multiple choice experiments were performed to compare attraction of *L. zonatus* nymphs and adults to the fruit of tomato, *Solanum lycopersicum* L.; satsuma mandarin; peach, *Prunus persica* (L.) Batsch; kumquat (*Fortunella* spp.); and lemon, *Citrus limon* (L.) Burm. F. Results of all three experiments clearly showed that tomato was the most preferred by both the nymphs and adults, with satsuma a distant second. Attraction to tomato and satsuma fruit was not due mainly to color but mediated by host volatile semiochemicals (kairomones). Developmental experiments with *L. zonatus* on satsuma fruit suggest that it is a suitable host that can maintain modest to high populations of the pest. Approximately 39 eggs were deposited per female on satsuma fruit with a hatch rate of 98%. Total developmental time from egg through the fifth nymphal stage was ≈50 d. High survivorship was recorded for all stages and ranged from 100% for the fourth instars to ≈89.1% for second instars. Cumulative survivorship from eggs through the fifth stage was 75.6%. Sex ratio (female:male) of emerged adults was 1:1.03, and female longevity (≈73 d) was significantly greater than male longevity (57 d). Other aspects of the developmental biology of *L. zonatus* on satsuma are described, and the results are discussed in relation to the field ecology of the pest.

**KEY WORDS** Satsuma mandarin, *Leptoglossus zonatus*, host preference, developmental biology

Leaffooted bugs (Hemiptera: Coreidae: *Leptoglossus* spp.) are polyphagous pests of various field, vegetable, and fruit crops in the United States (Hussey 1953, Allen 1969, Hall and Teetes 1982). *Leptoglossus zonatus* (Dallas) is an important emerging pest of a wide range of crops in the southern United States, including cotton, tomato, eggplant, peach, citrus, watermelon, corn, and pecan (Albrigo and Bullock 1977, Johnson and Allain 1998, Schaefer and Panizzi 2000, Buss et al. 2005). This species also was recently identified as a major pest of satsuma mandarin, *Citrus unshiu* Marcovitch, in the Gulf Coast region (Henne et al. 2003; Fadamiro et al. 2007, 2008). Feeding on satsuma fruit by nymphs and adults of *L. zonatus* or the closely related *Leptoglossus phyllopus* (L.) can result in premature color break and fruit drop, and render the fruit unmarketable (Henne et al. 2003). Damage also can reduce the soluble solids content of satsuma fruit (Y.F.X., unpublished data). In addition, *Leptoglossus* spp. can transmit a yeast disease pathogen (*Nemato-cera coryli*) to fruit, which causes dryness in the affected wedges of the fruit and produces bad fruit flavor (Henne et al. 2003).

Certain aspects of the life history of *L. zonatus* have been documented in some host crops (Panizzi 1989, Matrangolo and Waquil 1994, Jackson et al. 1995, Grimm and Somarriba 1999). Different developmental and survival rates were observed on various crops. For example, total developmental time at ≈30°C from second instars to final molt on maize, *Zea mays* L., was ≈42 d (Panizzi 1989), compared with ≈21 d on physic nut, *Jatropha curcas* L. (Grimm and Somarriba 1999). In southern Alabama, *L. zonatus* adults typically move from crop fields (e.g., cotton, tomato, and watermelon) into adjacent/nearby fruit orchards (e.g., satsuma and peach) in the fall when the fruits start to ripen. However, little is known about its host preference. Furthermore, it is not clear whether *L. zonatus* can complete its life cycle on satsuma. The development of an effective program for managing *L. zonatus* on satsuma requires knowledge of several aspects of its ecology and biology, including host preference and developmental biology. This study was conducted to determine 1) host preferences in *L. zonatus* by testing relative attraction of adults and nymphs to various fruit species in multiple choice tests; and 2) life history parameters of *L. zonatus* on satsuma fruit, including...
developmental times, survival rates, female fecundity, and adult longevity.

Materials and Methods

Rearing of *L. zonatus*. Adults of *L. zonatus* collected from satsuma orchards in southern Alabama in fall 2007 were used to start laboratory colonies, which were supplemented by adults collected from the field in 2008. Adults were reared in wooden sleeve cages (60 by 40 by 30 cm; five pairs per cage) with screened walls, on lima bean seedlings (three potted plants per cage) supplemented with fresh ripened satsuma fruit (three per cage), and water. The cages were checked daily to collect freshly laid eggs and to replace diet, as necessary. Lima bean seedlings were replaced monthly, whereas satsuma fruit were replaced biweekly. The rearing conditions were 25 ± 2°C, 50 ± 10% RH, and a photoperiod of 14:10 (L:D) h.

Host Preference in *L. zonatus*. Three separate multiple choice experiments were conducted to evaluate attraction of *L. zonatus* nymphs and adults to the fruit of the following crops: tomato, *Solanum lycopersicum* L. syn. *Lycopersicon lycopersicum*; satsuma mandarin; peach, *Prunus persica* (L.) Batsch; kumquat (*Fortunella spp.*); and lemon, *Citrus limon* (L.) Burm. F. In experiment 1, attraction of *L. zonatus* to the following five fruit treatments was compared: tomato (ripened, deep red color), satsuma (unripened, green), satsuma (ripened, orange), peach (ripened, yellow), and kumquat (ripened, yellow). In experiment 2, attraction of *L. zonatus* to the following four fruit treatments was compared: tomato (ripened, deep red color), satsuma (ripened, orange), peach (ripened, yellow), and lemon (ripened, yellow). Experiment 3 was conducted to test whether the attraction of *L. zonatus* to tomato (as observed in experiments 1 and 2) is related to color (deep red). For this experiment, green, unripened tomato was compared with the other three treatments tested in the second experiment. The tests were conducted in a wooden cage (60 by 40 by 30 cm) with screened (nylon screen net) walls under the above-mentioned conditions used for insect rearing. In experiment 1, the cage was divided into five sections (four corners and one middle section). One single fruit of each species/type (treatments) was placed in the bottom of one of the five sections. The experiment was replicated three times and the position of each treatment in the cage was determined randomly and rotated during each replication. *L. zonatus* of the following stages were tested: early instars (second–third), late instars (fourth–fifth), adult females, and adult males. For each stage, 10 individuals, previously starved for 8 h, were introduced into the center of the cage. The number of individuals on each fruit treatment 24 h later was counted.

In experiments 2 and 3, the cage was divided into four corner sections. One fruit of each species/type (treatments) was placed in the bottom of one of the four corners of the cage (one corner for each treatment). Ten individuals of late stage (fourth–fifth) nymphs or adult females, previously starved for 8 h, were introduced into the center of the cage. Each experiment was replicated eight times. The replication and treatment rotation schemes ensured that each treatment was located in each of the four corners of the cage twice. The number of individuals on each treatment was counted at 1, 6, 12, and 24 h after the insects were released into the cage.

Data obtained from the three experiments were first normalized by using the square-root transformation (√ x + 0.5). Significant differences in the number of individuals recorded on each treatment were established using one-way analysis of variance (ANOVA) followed by Tukey–Kramer honestly significant difference (HSD) comparison test (*P* < 0.05; JMP, version 7.01, SAS Institute 2007).

Development of *L. zonatus* on Satsuma Fruit. The life history parameters of *L. zonatus* on satsuma fruit were determined under laboratory conditions (25 ± 2°C, 50 ± 10% RH and a photoperiod of 14:10 [L:D] h), to evaluate the suitability of the fruit as host for the pest. Because it is difficult to obtain large number of eggs of *L. zonatus* on satsuma fruit in the laboratory, egg masses freshly laid on leaves of lima beans seedlings were collected daily and placed (with the leaves) into a glass jar (18 cm in length by 7.5 cm in diameter), where they were allowed to hatch and molt into second instars. Each observation consisted of 15–24 eggs, with five replications for a total of 94 eggs. The durations of the egg stage and the first stage nymphs (both on lima bean) were recorded. Newly molted second instars were reared on satsuma fruit in a glass jar (dimensions above) until they emerged as adults. The number and stage of the nymphs were recorded daily until adult emergence. From these data, the following life cycle parameters were computed: developmental time for each stage, total developmental time from egg to adult, stage-specific survivorship (survival of the different stages), and cumulative survivorship (calculated by dividing the total number of individuals observed at each stage [survivors] by the total number of eggs observed at the beginning of the experiment = 94 eggs). Newly emerged adults were sexed and the data used to calculate sex ratio, and then placed in pairs (male and female) in a glass jar (dimensions above) and reared on satsuma fruit at the above-stated conditions. Twelve pairs of adults were observed and the following data were recorded: preoviposition time, oviposition period, longevity, female fecundity (mean number of eggs oviposited), and egg hatch rate (percentage). Data were checked for normality and then subjected to one-way ANOVA followed by Tukey–Kramer HSD test (*P* < 0.05; JMP, version 7.01, SAS Institute 2007) to determine significant differences in developmental time among the different stages, and stage-specific survivorship. Significant sexual differences in adult longevity were established using Student’s *t*-test (*P* < 0.05; JMP, version 7.01, SAS Institute 2007).

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Results

Host Preference in *L. zonatus*. When simultaneously presented with the different fruit species (tomato, satsuma, peach, and lemon) in the three multiple choice experiments, nymphs and adults of *L. zonatus* were always found in greater numbers on tomato than on the other fruit species. In experiment 1, significantly higher numbers of *L. zonatus* of all stages were recorded on tomato than the other four treatments, 24 h after insects were released in the cage (early nymphs: $F = 18.14; \text{df} = 4, 10; P < 0.0001$; late nymphs: $F = 6.55; \text{df} = 4, 10; P = 0.007$; females: $F = 11.88; \text{df} = 4, 10; P = 0.0008$; males: $F = 6.10; \text{df} = 4, 10; P = 0.007$; Table 1). In general, no significant differences were recorded in the numbers of *L. zonatus* of all stages found on unripened versus ripened satsuma (Table 1).

In experiment 2, significantly more late stage nymphs were found on tomato (ripened, red) than on the remaining three fruit treatments (all ripened) at 6 h ($F = 16.55; \text{df} = 3, 28; P = 0.0001$), 12 h ($F = 46.4; \text{df} = 3, 28; P = 0.0001$), and 24 h ($F = 51.8; \text{df} = 3, 28; P = 0.0001$) after the insects were released in the cage (Fig. 1A). Similarly, significantly greater number of

<table>
<thead>
<tr>
<th>Stage/gender</th>
<th>Tomato (ripened)</th>
<th>Satsuma (unripened)</th>
<th>Satsuma (ripened)</th>
<th>Kumquat (ripened)</th>
<th>Peach (ripened)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early nymphs second–third</td>
<td>6.3 ± 0.3a</td>
<td>0.0 ± 0.0b</td>
<td>1.0 ± 0.6b</td>
<td>0.3 ± 0.3b</td>
<td>0.0 ± 0.0b</td>
</tr>
<tr>
<td>Late nymphs, fourth–fifth</td>
<td>2.7 ± 0.6a</td>
<td>1.3 ± 0.0ab</td>
<td>0.6 ± 0.3ab</td>
<td>1.3 ± 0.0ab</td>
<td>0.0 ± 0.0b</td>
</tr>
<tr>
<td>Females</td>
<td>2.7 ± 0.0a</td>
<td>0.0 ± 0.0b</td>
<td>0.7 ± 0.3b</td>
<td>0.3 ± 0.3b</td>
<td>0.0 ± 0.0b</td>
</tr>
<tr>
<td>Males</td>
<td>1.7 ± 0.0a</td>
<td>0.0 ± 0.0b</td>
<td>1.0 ± 0.0ab</td>
<td>0.3 ± 0.3ab</td>
<td>0.3 ± 0.3b</td>
</tr>
</tbody>
</table>

Means ± SE within the same row followed by different letters are significantly different ($P < 0.05$; Tukey–Kramer HSD test). For each stage/gender, 10 individuals were released per test and replicated three times.

![Fig. 1. Mean ± SE of *L. zonatus* nymphs (A) and adult females (B) recorded on different fruit species (all ripened) at different periods after they were released in a multiple-choice bioassay. Ten individuals were released per test and replicated eight times. Means for the same period having different letter in common are significantly different ($P < 0.05$; Tukey–Kramer HSD test).]
females were recorded on tomato (ripened, red) than on the remaining treatments at 6 h (F = 7.59; df = 3, 28; P = 0.0007), 12 h (F = 46.01; df = 3, 28; P = 0.0001), and 24 h (F = 84.15; df = 3, 28; P = 0.0001) after insect release (Fig. 1B). The next most attractive fruit species was satsuma, on which was recorded significantly greater numbers of nymphs and adult females compared with peach and lemon, at 24 h after insect release (Fig. 1A and B). In experiment 3, unripened (green) tomato was tested to determine whether the attraction of L. zonatus to tomato documented in experiments 1 and 2 is related to its deep red color. The results were generally similar for both the nymphs and adult females. The numbers of L. zonatus found on unripened tomato were significantly greater than the numbers found on each of the remaining three fruit treatments (all ripened) at 6 h (F = 25.9; df = 3, 28; P = 0.0001), 12 h (F = 12.03; df = 3, 28; P = 0.0001), and 24 h (F = 34.4; df = 3, 28; P = 0.0001) after insect release (adult females, Fig. 2B). No significant differences were recorded at 1 h after insect release. As recorded in the second experiment, the numbers of nymphs and adult females recorded on satsuma, 24 h after insect release, were significantly greater than the numbers recorded on peach or lemon.

Development of L. zonatus on Satsuma Fruit. Basic Description of the Stages. The egg masses of L. zonatus are golden brown, cylindrical and flattened, and they are laid in a straight line on the hosts. An egg mass contained 19.4 eggs on average (range, 15–32 eggs). Five nymphal stages (instars) were recorded. First-stage nymphs typically congregate under leaves (lima bean seedlings) or on the fruit (fruit crops), and feeding was initiated by the second-stage nymphs. The nymphs were bright red.

Immature Development and Survival. Under our rearing conditions an egg took 14.7 d to hatch. The mean developmental times for the first, second, third, fourth, and fifth instars were 3.5, 5.1, 5.9, 9.1, and 11.4 d, respectively (Table 2). Total developmental time from eggs through the fifth instars was \( \approx 50 \) d (Table 1). Development of early instars was significantly shorter compared with eggs and late instars (F = 490.11; df = 5, 465; P = 0.0001). High survivorship was recorded for all the stages but fourth instars had 100% survivorship. Cumulative survivorship from eggs through the fifth stage was 75.6% (Table 2).

Adult Survival and Reproduction. Mean preoviposition period was 13 d, and eggs were laid over \( \approx 39 \) d
Sorghum, including maize (Schaefer et al. 2000, Panizzi 2004); footed bugs are economic pests of important crops, feeding primarily on vegetative tissues such as shoots, but it also feeds occasionally on vegetable crops in the United States. Some species of plant bugs in the family Coreidae are known to feed on both fruit crops. Further studies including field tests are necessary to confirm our results and to identify the semiochemicals (kairomones) in tomato and satsuma that mediate the observed attraction of *L. zonatus* to both fruit species also occur in non-crop plants in the family Solanaceae, a family that also includes tomato, suggesting that the chemical cues mediating this attraction are shared by members of this plant family. If confirmed, highly attractive non-crop solanaceous plants could potentially be used as trap plants or sentinel monitoring plants for leaffooted bugs in fruit orchards and other economically important crops. Further studies including field tests are necessary to confirm our results and to identify the semiochemicals (kairomones) in tomato and satsuma that mediate the observed attraction of *L. zonatus* to both fruit crops.

This study demonstrates that *L. zonatus* can survive, develop, and reproduce on a diet consisting solely of satsuma fruit with no access to free water or supplementary food resources. Under our laboratory conditions, we recorded 39 eggs per female on satsuma fruit and an egg hatch rate of 98%. Total developmental

### Table 3. Life history parameters of *L. zonatus* on satsuma mandarin fruit under laboratory conditions

<table>
<thead>
<tr>
<th>Stage</th>
<th>Duration (d)</th>
<th>Total no. observed (n)</th>
<th>Stage-specific survivorship (%)</th>
<th>Cumulative survivorship (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>14.7 ± 0.2a</td>
<td>92</td>
<td>97.8</td>
<td>97.8</td>
</tr>
<tr>
<td>First instar</td>
<td>3.5 ± 0.1e</td>
<td>82</td>
<td>89.1</td>
<td>87.2</td>
</tr>
<tr>
<td>Second instar</td>
<td>5.1 ± 0.2d</td>
<td>78</td>
<td>95.1</td>
<td>82.9</td>
</tr>
<tr>
<td>Third instar</td>
<td>5.9 ± 0.2d</td>
<td>74</td>
<td>94.8</td>
<td>78.7</td>
</tr>
<tr>
<td>Fourth instar</td>
<td>9.1 ± 0.2c</td>
<td>74</td>
<td>100.0</td>
<td>78.7</td>
</tr>
<tr>
<td>Fifth instar</td>
<td>11.4 ± 0.7b</td>
<td>71</td>
<td>95.9</td>
<td>75.6</td>
</tr>
</tbody>
</table>

Means ± SE within the same column followed by different letters are significantly different (*P* < 0.05; Tukey-Kramer HSD test). Cumulative survivorship was calculated by dividing the total number of individuals observed at each stage (survivors) by the total number of eggs observed at the beginning of the experiment (94 eggs).

The results also showed that the relatively greater attraction of *L. zonatus* to tomato is not due mainly to color but may be mediated by chemical cues, possibly via olfaction or taste. Our observations also showed that tomato was almost always the first fruit species selected by both nymphs and adult females, further suggesting that olfaction is likely the main mechanism behind their preference for tomato. Host chemicals play a key role in mediating host preference in herbivorous insect species, most of which use host secondary chemicals (semiochemicals) as kairomones for host location (Duffey 1980, Brown 1984, Pasteels et al. 1988, Bowers 1990, Rank et al. 1998). Host biochemistry is dominant in the coevolution of plants and herbivorous insects and thus in determination of diet breadth (Ehrlich and Murphy 1988). Our results suggest that *L. zonatus* may initially select a host based on chemical characteristics rather than morphological characteristics. The similarities in the data obtained for unripened versus ripened fruit of tomato and satsuma suggest that the chemical cues mediating attraction of *L. zonatus* to both fruit species also occur in unripened fruits and that fruit maturity did not have a major effect on our results. Furthermore, results of an ongoing field study have demonstrated significant attraction and aggregation of *L. zonatus* on some non-crop plants in the family Solanaceae, a family that also includes tomato, suggesting that the chemical cues mediating this attraction are shared by members of this plant family. If confirmed, highly attractive non-crop solanaceous plants could potentially be used as trap plants or sentinel monitoring plants for leaffooted bugs in fruit orchards and other economically important crops. Further studies including field tests are necessary to confirm our results and to identify the semiochemicals (kairomones) in tomato and satsuma that mediate the observed attraction of *L. zonatus* to both fruit crops.

### Table 2. Life history parameters of *L. zonatus* (eggs and nymphs) on satsuma mandarin fruits under laboratory conditions

<table>
<thead>
<tr>
<th>Gender</th>
<th>Parameter</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Preoviposition  (d)</td>
<td>13.9 ± 1.1</td>
<td>11.0–15.0</td>
</tr>
<tr>
<td></td>
<td>Oviposition (d)</td>
<td>38.3 ± 4.5</td>
<td>12.0–64.0</td>
</tr>
<tr>
<td></td>
<td>Feecundity (eggs per female)</td>
<td>38.3 ± 3.9</td>
<td>0.0–44.0</td>
</tr>
<tr>
<td></td>
<td>Egg hatch rate (%)</td>
<td>98.3 ± 1.7</td>
<td>91.7–100.0</td>
</tr>
<tr>
<td>Female</td>
<td>Longevity (d)</td>
<td>49.3 ± 2.0</td>
<td>41.0–53.0</td>
</tr>
<tr>
<td>Male</td>
<td>Longevity (d)</td>
<td>72.6 ± 8.0 a</td>
<td>19.0–125.0</td>
</tr>
</tbody>
</table>

Significant sexual difference in longevity established using a *t*-test (*P* < 0.05).
time from egg through the fifth stage was $\approx 50$ d. High survivorship was recorded for all the immature stages, ranging from 100% for fourth instars to 89.1% for first instars. A few studies have examined the development of *L. zonatus* on other crops or artificial diets (Panizzii 1989, Matrangolo and Waquil 1994, Jackson et al. 1995, Grimm and Somarriba 1999). Many of our data are similar to those reported in the above-mentioned studies, but with some key differences. For example, the total developmental time for the nymphal stages recorded on satsuma in this study (36.4 d) is much longer than the 28.7 d on maize and 31.6 d on sorghum reported by Matrangolo and Waquil (1994) or the 25.6 d recorded on physic nut (Grimm and Somarriba 1999). However, the 23.8% mortality rate that we recorded for the nymphal stage on satsuma was much lower than the 53.8 and 55.1% recorded on maize and sorghum, respectively (Matrangolo and Waquil 1994), or the 59.7% mortality recorded on physic nut (Grimm and Somarriba 1999). The differences between our data and those reported previously may be related to differences in experimental conditions and may not truly reflect the suitability of the different crops as hosts for *L. zonatus*.

Female and male *L. zonatus* fed on satsuma fruit lived on average for $\approx 73$ and 57 d, respectively. Our data on adult longevity are similar to the $\approx 71$ and 54 d recorded for females and males, respectively, on sorghum (Matrangolo and Waquil 1994) but shorter than the $\approx 87$ and 84 d recorded for females and males, respectively, on physic nut (Grimm and Somarriba 1999). However, the $\approx 39$ eggs deposited per female on satsuma was less than the 96 eggs laid on sorghum (Matrangolo and Waquil 1994), the 229 eggs on physic nut (Grimm and Somarriba 1999), and the 345 eggs deposited on a meridic diet (Jackson et al. 1995).

Altogether, these results suggest that satsuma fruit is an excellent host for development of *L. zonatus* but not an optimal host for oviposition. The reduced oviposition on satsuma relative to those reported on other crops may be related to the waxy texture of the fruit or to other yet unknown factors. Further studies are necessary to investigate the basis for the reduced oviposition by *L. zonatus* on satsuma and to compare oviposition and development on other fruit crops, such as tomato and peach. Despite the lower number of eggs deposited on satsuma fruit in this study, our data, including the near 1:1 sex ratio of emerged adults recorded in this study, suggest that high populations of biologically fit *L. zonatus* can be maintained on this fruit crop.

Acknowledgments

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