

# The Effect of Seasonal Changes on *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) in Hawaii

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**The effect of seasonal changes in temperature and photoperiod on the interaction of *Nezara viridula* (Hemiptera: Pentatomidae) and its egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae) was investigated in the laboratory. We found no evidence of reproductive diapause in *N. viridula* under simulated Hawaiian summer and winter conditions. Further, although “diapause” coloration was obtained in the laboratory, it was not correlated with reproductive status. Studies of the survival of *T. basalis* provided with honey under the same simulated conditions showed that under summer conditions, only 2.1% of the female and 13.5% of the male population were still alive by 60 days. When provided with *N. viridula* egg masses at 30 days, 79.4% of the eggs were parasitized in a 3-h period. Under winter conditions, 54.3, 28.3, and 14.5% of the females were alive at 30, 60, and 90 days after adult emergence. When provided with *N. viridula* egg masses at 30, 60, and 90 days for 3 h, 57.6, 32.8, and 47.1% of the eggs were successfully parasitized. These studies suggest the limiting factor in the interaction of *T. basalis* and *N. viridula* is not reproductive diapause, but instead the ability of *T. basalis* to survive summer conditions.** © 2002 Elsevier Science

**Key Words:** *Nezara viridula*; *Trissolcus basalis*; biological control; diapause.

## INTRODUCTION

The southern green stinkbug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), was first found in Hawaii on the island of Oahu in 1961 and by 1963 had spread to all seven of the major islands (Davis, 1964). It rapidly became a pest of macadamia nuts and certain vegetable and ornamental crops (Mitchell *et al.*, 1965).

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Biological control efforts began in 1963 and releases of natural enemies resulted in the establishment of *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) and *Trichopoda pilipes* (F.) (Diptera: Tachinidae) (Davis, 1964, 1967). Following the release and establishment of these natural enemies, parasitism rates were considered high enough that the effort was considered a success and releases were discontinued in 1966 (Davis, 1967).

In reviewing the studies of Davis (1964, 1967), it becomes obvious that parasitism by *T. basalis* was different among islands. For example, on the island of Hawaii, parasitism from *T. basalis* was never reported to be higher than 55%, while parasitism on Oahu was considered to be virtually 100% (Davis, 1967). In the late 1980s, macadamia growers on the island of Hawaii complained that damage from *N. viridula* was excessive. Jones and Caprio (1992) confirmed that nut damage was up to 60% in some locations and began studies to determine the effectiveness of *T. basalis*. Jones (1995) found that in macadamia orchards on the island of Hawaii that *T. basalis* parasitism was extremely low and suggested that ants and generalist predators played a larger role in population regulation than *T. basalis*. Work in Australia by Clarke and his co-workers (Clarke, 1990; Clarke and Walter, 1990; Clarke, 1992a,b) also found that that *T. basalis* is not as efficient as previously thought. Clearly, biological control of *N. viridula* is not the “landmark” example of success in either Hawaii or Australia as suggested by Caltagirone (1981).

Studies in Australia suggest that the low efficiency of *T. basalis* is a combination of reduced survival during periods without stinkbug eggs (Clarke and Walter, 1993) when *N. viridula* is either in reproductive diapause or when it is simply not laying eggs because host plants are unsuitable (Velasco and Walter, 1993). Clarke and Walter (1993) found that under a winter temperature regime (in Brisbane, SE Queensland) no significant mortality occurred between 50 and 124 days

after emergence if *T. basalis* was provided with honey and water. Further, if *N. viridula* egg masses were provided after  $\approx 150$  days, surviving females were able to successfully parasitize them. However, their studies on survival throughout the summer egg-free period suggest that summer temperatures may be extremely important. They found that 90% of the *T. basalis* population exposed to summer temperatures (mean high temperature  $>25^{\circ}\text{C}$ ) died by 55 days, with 80% of the mortality occurring in the first 30 days (Clarke and Walter, 1993). Unfortunately, the studies of Clarke and Walter (1993) were performed under uncontrolled (ambient) temperatures and do not provide sufficient information on the tolerance of *T. basalis* to summer temperatures to be useful in other areas.

The ability of *T. basalis* to survive egg-free periods and the importance of diapause on *N. viridula* egg production in Hawaii are unknown. Early reports indicate that *N. viridula* undergoes continuous reproduction in Hawaii (Mitchell *et al.*, 1965; Nishida, 1966), but we have collected adult *N. viridula* with "diapause" or russet coloration during the fall (V. P. Jones, unpublished). Unfortunately, work in Australia has shown that the color change is not a good indicator of diapause and that several individuals with "diapause" coloration were laying eggs and, when dissected, showed fully mature ovaries (Seymour and Bowman, 1994). However, studies in other areas show that the critical photoperiod for diapause induction is between 10 and 12 h of light (Ali and Ewiess, 1977; Todd, 1989), which is within the typical range of daylight found in Hawaii (11–13 h daylight; USNO, 1993). These facts, combined with the broad host range of *N. viridula* and the relatively mild winter temperatures common in Hawaii may result in at least part of the population entering reproductive diapause. Even if only a proportion of the female population enters diapause, it may have a crucial effect on *T. basalis* population dynamics because if there is an egg-free period, then *T. basalis* must be able to survive on alternate prey or on pollen, nectar, or honeydew sources.

The studies reported here were initiated to determine if *N. viridula* undergoes reproductive diapause in the relatively mild climate of Hawaii and if Hawaiian *T. basalis* were able to survive these periods and successfully parasitize eggs laid after its succession. In addition, we investigated the accuracy of using the russet coloration of adult females in predicting *N. viridula* reproductive status under Hawaii conditions.

## MATERIALS AND METHODS

### *Diapause Studies on N. viridula*

Diapause of *N. viridula* was determined in laboratory studies so that we could control both temperature and photoperiod in growth chambers. Winter condi-

tions were set at  $23.8^{\circ}\text{C}$  (day) and  $15^{\circ}\text{C}$  (night) with an 11:13 (L:D) h photophase. Summer conditions were set at  $28.8^{\circ}\text{C}$  (day) and  $23.3^{\circ}\text{C}$  (night) with a 13:11 (L:D) h photophase. Temperature and photoperiods were set by examining the mean temperature and photoperiod (United States Naval Observatory, 1993) recorded at the UH Kona Research Station, Kealahou, Hawaii, during the months of December and June during the years 1988–1992. Daily mean high and low temperatures were obtained from the same years from the journal *Climatic Data*.

Colonies of *N. viridula* were maintained in the laboratory on a standard diet of green beans and peanuts (Harris and Todd, 1981). We collected egg masses from the colony the day they were laid and placed one to two egg masses into a  $30.5 \times 30.5 \times 30.5$  cm wire-screened cage. A total of six cages were setup in the high-temperature regime and six in the low-temperature regime. All insects were collected as they died in each experiment, frozen, and later dissected to determine reproductive status. To prevent bias caused by early death in determination of mating status, maturity, and the other indicators of physiological state, insects that died before the first eggs were found by dissection in the oviduct were not used in the analysis. We used the classification system of Kiritani (1963) to determine the mating status, maturity, the size of the fat body (small, medium, or large), size of the fat body cells (small or large), adult color (green or russet), presence of eggs in the oviducts, and the number of eggs present.

In order to have a baseline indication of the percentages of the adult population that would be mated or mature, we ran a preliminary experiment with an extremely short photoperiod (8 L:16 D) and a constant temperature of  $21.6^{\circ}\text{C}$ . For this experiment, we only set up a single cage, but used all the same methods as above.

Data were analyzed by *G* tests (SAS, 2000) to determine if there were significant differences in the variables investigated for the two environmental extremes. For egg-bearing females only, the number of eggs present was compared between environmental conditions using a nested ANOVA where the color morph was nested within the environmental condition (SAS, 2000). For the males, only the percentage mature in the different environmental conditions was analyzed using *G* tests. The effect of the color morph was examined by combining the data for both the summer and winter conditions and examining the variables (mating status, etc.) using *G* tests.

### *Laboratory Studies of T. basalis Longevity and Diapause*

We also performed studies on the effect of temperature and photoperiod on *T. basalis*. We used the same temperature and photoperiod conditions described in

**TABLE 1**  
Effect of Environmental Conditions on *N. viridula* Physiological Status

Characteristic examined	Sex	Environmental condition		<i>G</i> <sup>d</sup>	<i>P</i> > <i>G</i>
		Summer <sup>a</sup> ( <i>N</i> ) <sup>b</sup>	Winter <sup>c</sup> ( <i>N</i> )		
% Sexually mature	Female	70.9 (55)	63.8 (58)	0.65	0.42
	Male	89.8 (59)	93.1 (73)	0.47	0.49
% Females mated		87.0 (54)	94.9 (59)	2.12	0.14
% Mature females with eggs		100 (39)	97.3 (37)	1.45	0.23
% With full stomach	Female	54.5 (44)	43.9 (41)	0.96	0.33
	Male	68.9 (45)	67.3 (55)	0.03	0.86
% With normal size fat body cells	Female	100 (38)	59.4 (32)	24.0	<0.0001
	Male	100 (48)	86.5 (52)	9.6	0.002

<sup>a</sup> Temperature 28.8°C:23.3°C (L:D); photoperiod 13 L:11 D.

<sup>b</sup> Sample size in parenthesis.

<sup>c</sup> Temperature 23.8°C:15°C (L:D); photoperiod 11 L:13 D.

<sup>d</sup> All comparisons are 1 *df*.

our *N. viridula* diapause experiments. A *T. basalis* colony was started from parasitized *N. viridula* egg masses found in several locations on the island of Oahu and reared in 41 × 41 × 41 cm cages on *N. viridula* egg masses. The colony was maintained in the laboratory at ≈24°C with a 14:10 (L:D) h photoperiod. For the experiments, we placed *N. viridula* egg masses in the *T. basalis* colony for 24 h and then removed the eggs and placed them randomly in cages in either the summer or the winter conditions. After emergence, *T. basalis* was provided with honey streaked on the inside of the cages for 30 days, but no host eggs. At 30 days parasitoids were provided with a single *N. viridula* egg mass for 3 h to determine if they could successfully parasitize the egg mass after deprivation. A single *N. viridula* egg mass was again provided at 60 days, and at 90 days, five egg masses were added for a 48-h period. All egg masses were held for parasitoid emergence. *Trissolcus basalis* mortality was recorded daily over the experimental period. Five trials were conducted for the summer conditions and four trials for the winter conditions. A total of 141 males and 191 females were followed in the summer conditions and 121 males and 146 females in the winter conditions.

The survival data were graphed as percentage survival versus time for both males and females at the two different temperature/photoperiods. The mean percentage survival at 30 and 60 days between the two environmental conditions and sexes were compared using *G* tests (SAS, 2000).

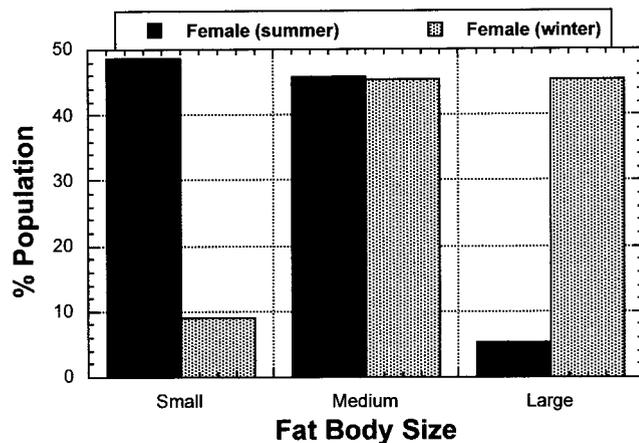
## RESULTS

### Diapause Studies on *N. viridula*

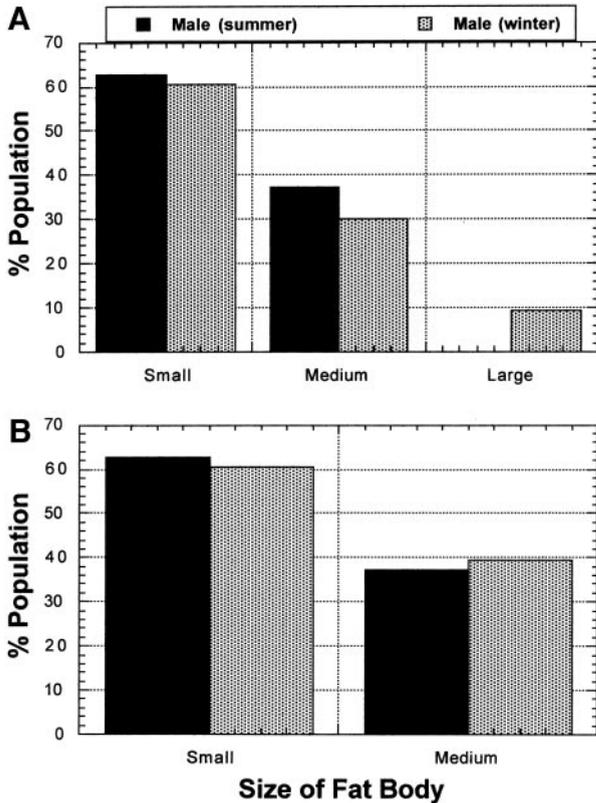
The baseline diapausing population was found to have 73.7% of the females immature (*n* = 19), but 52.6% were mated by 30 days after the first eggs were

detected. Six of the immature females were mated. Male mortality was high. Only four males survived to the end of the experiment, but all were sexually mature.

No significant differences were detected between females reared under the summer or winter conditions in the percentage of females sexually mature, percentage mated, percentage of sexually mature females with eggs, or the percentage with a full stomach (Table 1). However, the percentage of normal size fat body cells was significantly lower in the winter (59.4%) than in the summer (100%) conditions (Table 1). In addition, there were significant differences in the proportion of insects that showed large, medium, or small amounts of fat body related to temperature (*G* = 23.0, *df* = 2, *P* < 0.001) (Fig. 1). Only 5.4% of the females reared under summer conditions had large amounts of fat body compared to 45.4% in those reared under the winter conditions. Conversely, 48.6% of the females



**FIG. 1.** Effect of simulated winter and summer conditions on size of *N. viridula* female fat body.



**FIG. 2.** Effect of simulated winter and summer conditions on size of *N. viridula* male fat body. (A) Using small, medium, and large categories. (B) Combining large and medium categories to eliminate problem with having more than 20% of the cells with expected counts <5.

reared under the summer conditions had small amounts of fat body, whereas 9.1% of the females reared under the winter conditions had small amounts of fat body. The analysis of the number of eggs present in females showed there were no significant effects from environmental condition [ $F(1, 72) = 2.91, P = 0.09$ ].

Examination of the males showed that there were no significant differences in the percentage sexually ma-

ture or the percentage with a full stomach (Table 1). However, the percentage of males with normal size fat body cells was significantly higher under the summer conditions (100%) than under the winter conditions (86.5%) (Table 1). Analysis of the amount of fat body also showed significant differences in the proportions with high, medium, and low amounts of fat body ( $G = 6.3, df = 2, P = 0.04$ ) (Fig. 2a). However, examining the data shows that the statistical difference is a result of finding no males with large amounts of fat body in the summer conditions and only five in the winter conditions. The low sample size in the large category affects the  $G$  test (SAS, 2000), so we combined the medium and large categories and reran the analysis. When this was done, there were no significant differences found between the different environmental conditions ( $G = 0.06, df = 1, P = 0.81$ ) (Fig. 2b).

#### *Suitability of Color as a Predictor of Diapause*

Only the percentage of males with a full stomach showed significant differences between the two different color morphs (Table 2). Examination of the number of eggs produced by egg-producing females between the two color morphs also showed no significant differences [ $F(1, 72) = 2.91; P = 0.09$ ].

#### *Laboratory Studies of T. basalis Longevity and Diapause*

The winter conditions resulted in consistently higher *T. basalis* survival than the summer conditions for both sexes (Fig. 3). Analysis of the survival at 30 days showed that females experienced significantly higher survival under the winter conditions [54.3 (winter) versus 26.2% (summer);  $G = 27.1, df = 1, P < 0.0001$ ], but males did not [60.2 (winter) versus 51.1% (summer);  $G = 2.1, df = 1, P = 0.15$ ]. When the data at 60 days were tested, we found significantly lower survival in both females [28.3 (winter) versus 2.1% (summer);  $G = 51.9, df = 1, P < 0.0001$ ] and males [40.7 (winter) versus 13.5% (summer);  $G = 24.7, df = 1, P < 0.0001$ ] under the summer conditions.

**TABLE 2**

Effect of Color Morph on *N. viridula* Physiological Status

Characteristic examined	Sex	Green <sup>a</sup>	Russet <sup>a</sup>	$G^b$	$P > G$
% Sexually mature	Female	66.3 (59)	70.8 (24)	0.18	0.67
	Male	91.0 (111)	95.2 (24)	0.47	0.49
% Females mated		89.9 (89)	95.8 (24)	0.97	0.33
% Mature females with eggs		98.3 (59)	100 (17)	0.51	0.47
% With full stomach	Female	89.4 (67)	44.4 (18)	0.23	0.63
	Male	63.9 (83)	88.2 (17)	4.45	0.03
% With normal size fat body cells	Female	83.3 (54)	75.0 (16)	0.54	0.46
	Male	94.1 (85)	86.7 (15)	0.91	0.34

<sup>a</sup> Sample size in parenthesis.

<sup>b</sup> All tests with 1 *df*.

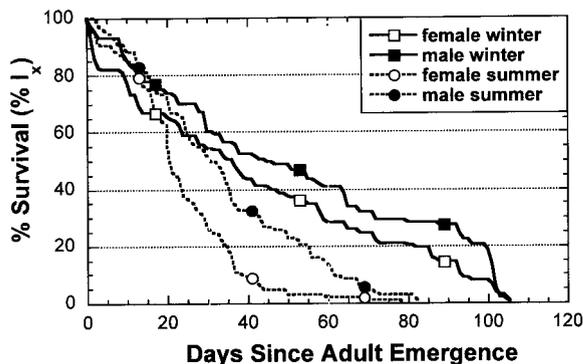


FIG. 3. Survival of *T. basalis* under simulated summer and winter conditions.

Despite the low survival at 30 days in the summer conditions, parasitism averaged  $79.4\% \pm 21.5$ , but by 60 days was unobtainable in four of the five trials because complete female mortality had occurred by that time. For the one trial where mortality was not complete, we had a mean parasitism of 91%. Under the winter conditions, parasitism was  $57.6\% \pm 38.6$  and  $32.8\% \pm 47.1$  at 30 and 60 days, respectively. Parasitism at  $\geq 90$  days was successful in all five trials, averaging  $47.1 \pm 22.3\%$  at 90 days and decreasing slowly until complete female mortality occurred.

## DISCUSSION

These data suggest that *N. viridula* populations do not undergo reproductive diapause under the relatively mild climatic and photoperiod changes seen in Hawaii. We did see changes in the fat body and fat body cells, but none of the other indicators suggested diapause occurred. In contrast, the preliminary study with a 8 L:16 D photophase showed that the majority of the individuals did not become reproductively mature and eggs were only found in 3 of the 19 females. Specimens showing "diapause" coloration differed from nondiapause coloration only in the percentage of males with a full stomach, but not in mating status, sexual maturity, percentage of mature females with eggs, or in size of fat body cells or amount of fat body. Thus our study and that of Seymour and Bowman (1994) both agree that the "diapausing" coloration is not a reliable predictor of diapause.

Our data and that of Clarke and Walter (1993) on *T. basalis* longevity agree that summer conditions are a likely limiting factor for biological control of *N. viridula*. Surprisingly, the mortality of *T. basalis* under summer conditions at 30 and 55 days was nearly identical between the two studies. However, Clark and Walter (1993) found that no significant mortality of *T. basalis* occurred in the winter conditions between 50 and 124 days. In contrast, we found that *T. basalis* mortality increased at a relatively constant rate

throughout the life of both males and females and all individuals were dead by 106 days after adult emergence. Under both the summer and winter conditions, *T. basalis* females had lower survival than males at any given time.

The relatively high mortality of females at the summer temperatures suggests that *T. basalis* may not be able to regulate populations of *N. viridula* that are at low levels and thus either spatially or temporally patchy. If *T. basalis* is not able to find *N. viridula* egg masses within a comparatively short period of time, then *T. basalis* populations drop drastically and the efficiency of *N. viridula* population regulation would decrease markedly. The relatively long developmental period of *N. viridula* under these temperatures ( $\approx 60$  days) means that unless *T. basalis* is well synchronized with *N. viridula* populations or has alternate hosts to keep its population levels high, then some other factor must be regulating the *N. viridula* populations. In macadamia orchards on the island of Hawaii, we have found no other pentatomid bug over the past 10 years (V. P. Jones, unpublished) and egg predation is consistently high (Jones, 1995; V. P. Jones, unpublished). These facts suggest that suitable (and uneaten) egg masses in macadamia nut orchards are rare and may act as a limiting factor in *T. basalis* reproduction and hence its ability to regulate *N. viridula*.

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## REFERENCES

- Ali, M., and Ewiess, M. A. 1977. Photoperiodic and temperature effects on rate of development and diapause in the green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae). *Z. Ang. Ent.* **84**, 256–264.
- Caltagirone, L. E. 1981. Landmark examples in classical biological control. *Annu. Rev. Entomol.* **26**, 213–232.
- Clarke, A. R. 1990. The control of *Nezara viridula* L. with introduced egg parasitoids in Australia: A review of a "Landmark" example of classical biological control. *Aust. J. Agric. Res.* **41**, 1127–1146.
- Clarke, A. R. 1992a. Current distribution and pest status of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in Australia. *J. Aust. Ent. Soc.* **31**, 289–297.
- Clarke, A. R. 1992b. "Investigations into the Biological Control of *Nezara viridula* (L.) by the Egg Parasitoid *Trissolcus basalis* (Wollaston) in Eastern Australia." Ph.D. dissertation, Univ. of Queensland, Brisbane.
- Clarke, A. R., and Walter, G. H. 1990. *Trissolcus basalis* as a biological control agent of *Nezara viridula* in south east Queensland. In Proc. 6th Aust. Soybean Res. Workshop (H. Rose, Ed.), pp. 71–73. QDPI Hermitage Research Station, Warwick.

- Clarke, A. R., and Walter, G. H. 1993. Biological control of green vegetable bug (*Nezara viridula* (L.)) in Eastern Australia: Current status and perspectives. In "Pest Control and Sustainable Agriculture" (S. A., Corey, D. J. Dall, and W. M. Milne, Eds.), pp. 223–225. CSIRO Div. Entomology, Canberra.
- Davis, C. J. 1964. The introduction, propagation, liberation and establishment of parasites to control *Nezara viridula* variety *smaragdula* (Fabricius) in Hawaii (Heteroptera: Pentatomidae). *Proc. Hawaii Entomol. Soc.* **18**, 369–375.
- Davis, C. J. 1967. Progress in the biological control of the southern green stinkbug, *Nezara viridula* variety *smaragdula* (Fabricius) in Hawaii (Heteroptera: Pentatomidae). *Mushi*. **39**, 9–16.
- Harris, V. E., and Todd, J. W. 1981. Rearing the southern green stink bug, *Nezara viridula*, with relevant aspects of its biology. *J. Ga. Entomol. Soc.* **16**, 203–210.
- Jones, V. P. 1995. Reassessment of the role of predators and *Trissolcus basalus* in biological control of southern green stinkbug (Hemiptera: Pentatomidae) in Hawaii. *Biol. Contr.* **5**, 566–572.
- Jones, V. P., and Caprio, L. C. 1992. Damage estimates and population trends of insects attacking seven macadamia cultivars in Hawaii. *J. Econ. Entomol.* **85**, 1884–1890.
- Kiritani, K. 1963. The change in reproductive system of the southern green stink bug, *Nezara viridula*, and its application to forecasting of the seasonal history. *Jpn. J. Appl. Ent. Zool.* **7**, 327–337.
- Mitchell, W. C., Warner, R. M., and Fukunaga, E. T. 1965. Southern green stinkbug, *Nezara viridula* (L.), injury to macadamia nut. *Proc. Hawaiian Entomol. Soc.* **19**, 103–109.
- Nishida, T. 1966. Behavior and mortality of the southern stinkbug *Nezara viridula* in Hawaii. *Res. Pop. Ecol.* **8**, 78–88.
- SAS. 2000. "JMP Statistics and Graphics Guide," version 4. SAS Institute Inc, Cary, NC.
- Seymour, J. E., and Bowman, G. J. 1994. Russet coloration in *Nezara viridula* (Hemiptera: Pentatomidae): An unreliable indicator of diapause. *Environ. Entomol.* **23**, 860–863.
- Todd, J. W. 1989. Ecology and behavior of *Nezara viridula*. *Annu. Rev. Entomol.* **34**, 273–292.
- United States Naval Observatory. 1993. "The Astronomical Almanac." U.S. Government Printing Office, Washington, DC.
- Velasco, L. R. I., and Walter, G. H. 1993. Influence of temperature on survival and reproduction of *Nezara viridula* (L.) (Hemiptera: Pentatomidae). *J. Aust. Ent. Soc.* **32**, 225–228.