ECOLOGY, BEHAVIOR AND BIONOMICS

Nymphal and Adult Performance of Genetically Determined Types of *Nezara viridula* (L.) (Heteroptera: Pentatomidae), under Different Temperature and Photoperiodic Conditions

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Desempenho de Ninfas e de Adultos de Tipos Geneticamente Determinados de *Nezara viridula* (L.) (Heteroptera: Pentatomidae) sob Diferentes Condições de Temperatura e Fotoperíodo

RESUMO - O percevejo verde, *Nezara viridula* (L.), possui vários tipos determinados geneticamente sendo três mais comuns no Brasil: G (f. *smaragdula* – corpo verde), O (f. *torquata* – corpo verde com os lobos laterais e medianos da cabeça e margem anterior do pronoto de cor amarela), e Y (f. *aurantiaca* – corpo amarelo ou alaranjado). Estudou-se a performance das ninfas e dos adultos desses tipos a 15ºC/10hL, 22ºC/12hL e 29ºC/14hL. A mortalidade das ninfas dos três tipos a 15ºC/10hL foi alta (ca. 80%), em especial para o tipo G (98%); a 22ºC/12hL, a maior mortalidade (55%) ocorreu para o tipo G, e a 29ºC/14hL para o tipo Y (65%). Em geral, à medida que aumentou a temperatura e o fotoperíodo as ninfas completaram o ciclo mais rápido. A longevidade dos adultos do tipo G decresceu de ca. 88 dias a 15ºC/10hL a ca. 57 dias a 22ºC/12hL e a 29ºC/14hL; para o tipo O a longevidade variou de ca. 81 dias a 22ºC/12hL a ca. 55 dias a 29ºC/14hL; e para o tipo Y a longevidade foi a menor, apresentando redução drástica (< 20 dias) nos extremos das temperaturas/fotoperíodos. Os tipos G e Y não se reproduziram a 15ºC/10hL, e o Y não se reproduziu a 29ºC/14hL; o tipo O reproduziu-se nas três temperaturas/fotoperíodos. Esses resultados demonstram que o tipo O é mais adaptado às temperaturas/fotoperíodos menores o que explica a sua maior ocorrência no extremo sul do Brasil.

PALAVRAS-CHAVE: Biologia, tipo genético, percevejo verde

ABSTRACT - The southern green stink bug, *Nezara viridula* (L.), has several genetically determined types, three being the most common in Brazil: G (f. *smaragdula* – green body), O (f. *torquata* – green body with lateral and median lobes of the head and anterior margin of the pronotum yellow), and Y (f. *aurantiaca* – gold or orange body). Nymphal and adult performance of these types was studied at 15ºC/10hL, 22ºC/12hL, and 29ºC/14hL. Mean total nymph mortality in all types at 15ºC/10hL was high (ca. 80%), especially for type G (98%); at 22ºC/12hL, the greatest mortality (55%) occurred in type G, and at 29ºC/14hL in type Y (65%). In general, at combinations of higher temperatures and longer photoperiods, nymphs developed faster. Adult longevity of type G decreased from ca. 88 days at 15ºC/10hL to ca. 57 days at 22ºC/12hL and 29ºC/14hL; for type O, adult longevity varied from ca. 81 days at 22ºC/12hL to ca. 55 days at 29ºC/14hL; type Y showed the shortest lifespan, in particular at the extremes of temperatures/photoperiods (< 20 days). Types G and Y did not reproduce at 15ºC/10hL, and type Y did not reproduce at 29ºC/14hL; type O reproduced at all three abiotic conditions. These results demonstrate that type O is the most adapted to the cooler temperature and shorter photoperiod, which explains its greater abundance in south Brazil.

KEY WORDS: Biology, genetical type, southern green stink bug

The southern green stink bug, *Nezara viridula* (L.) is polyphagous, feeds on a wide array of plants, both wild and cultivated, and has a worldwide distribution (Panizzi *et al.* 2000).

N. viridula is known to be polymorphic and several genetically determined types have been described primarily from the Oriental Region (Kiritani & Yukawa 1963). Among them, three basic types are known to occur in Brazil, which are: G (f. *smaragdula* – entirely green body), O (f. *torquata* – green body with yellow lateral and median lobes of the head and anterior margin of the pronotum), and Y (f. *aurantiaca* – entirely gold or orange body). In addition, two other types derived from the basic types G and O, having a yellowish-green background instead of green, were recently found; G-yellowish type was obtained from the field in Londrina, PR (latitude 23º18' S), and O-yellowish type was obtained in the laboratory (Vivan & Panizzi 2005). It is known that different morphs intermediate between the basic types of *N. viridula* might be obtained in the laboratory by crossing (Ohno & Alam 1992), but this also occurs in the field.

Some types of *N. viridula* are known to be better adapted to unfavorable abiotic conditions due to their variable genetic composition, and this is an important factor that contributes to the abundance of the populations (Kiritani 1971).

The biology of *N. viridula* has been studied worldwide (references in Panizzi 1997 and Panizzi *et al*. 2000). However, few data on the performance of nymphs and adult of different types, related to the variable conditions of temperature and photoperiod, are available. Therefore, studies were conducted to evaluate the effect of different temperatures and photoperiods on the biology of nymphs and adults of the three basic types of *N. viridula* referred above.

Material and Methods

Laboratory studies were conducted during January-May 2002. Adults of *N. viridula* were field collected in Londrina, PR (types G and Y - latitude 23º 18' S) and in Cruz Alta, RS (type O - latitude 28º 39' S). Parents of each type crossed in the laboratory and the F1 generations resulting from the crosses were used for the tests. Egg masses were put singly in Petri dishes (9.0 x 1.5 cm), lined with filter paper, and kept in an environmental chamber at 25 ± 1ºC, 65 ± 5% RH, and 14hL.

Nymphal Study. During the first day of the 2nd instar (1st instars are gregarious and do not feed), nymphs of the G, O, and Y types were separated, each placed singly in a petri dish as described above and provided with immature soybean pods [*Glycine max* (L.) Merrill; cv. BR-37]. The dishes were then placed in environmental chambers with the following conditions of temperature and photoperiod: 15ºC/10hL, 22ºC/12hL, and 29ºC/14hL (temperatures varied within 1ºC). These conditions were selected in order to roughly simulate temperatures and photoperiods that occur in the field during certain times of the year, according to the different seasons. The number of nymphs used at each temperature/photoperiod was, respectively, 56, 56, and 58 (type G); 83, 83, and 76 (O); and 11, 36, and 11 (Y).

Nymphs were observed daily, and the food replaced every three days. Molting to the next instars and nymph mortality were recorded. The time of molt, percentage nymphal mortality, and mean (± SEM) nymphal developmental time were calculated.

Adult Study. Pairs of *N. viridula* were selected at the adult emergence date and placed each in plastic boxes (11 x 11 x 3.5 cm) covered with a lid, and lined with filter paper. Adults were fed as described above. The boxes were then placed in environmental chambers with the same three sets of temperature and photoperiodic conditions as used for the nymphs. The number of pairs used at the different temperatures was, respectively, 12, 12, and 22 (type G); 6, 16, and 12 (O); and 4, 16, and 2 (Y). Adults were observed daily, and the food was replaced every three days. Adult survivorship and fecundity were recorded, and the mean (± SEM) adult longevity (days) and fecundity (number of egg masses and number of eggs per female) were calculated.

Statistics. Data on nymph mortality at each temperature and for each type were submitted to analyses of regression; data on nymph developmental time, on adult longevity (days), and on adult fecundity (number of egg masses and number of eggs per female) were submitted to analysis of variance (ANOVA), and means were compared using the Tukey test ($P < 0.05$). Analyses were performed using the SAS program (SAS Institute 2001).

Results

Nymphal Survivorship. Survivorship of *N. viridula* nymphs varied in different types and different temperature/ photoperiod combinations. In general, nymph mortality decreased with the increase in temperature, with this negative correlation showing high values for the coefficients of determination R^2 for types *smaragdula* (G) and *torquata* (O), but not for type *aurantiaca* (Y) (Fig. 1). Type G showed greater total nymphal mortality of ca. 98% and 55% at 15ºC/ 10hL and at 22ºC/12hL, respectively, which decreased to ca. 30% at 29ºC/14hL (Fig. 1). In types O and Y, nymphal mortality was ca. 82 and 90%, respectively, at the lower temperature and shorter photoperiod, and decreased to ca. 40% at intermediate conditions. However, at the combination of the highest temperature and longest photoperiod, types G and O had mean total mortalities of ca. 35%, while in type Y this value was raised to ca. 65%.

In general, the highest mortality (ca. 70%) occurred during the 2nd instar at 15ºC/10hL. At 22ºC/12hL it was lower with a range of mortality ca. 15-35%. At 29ºC/14hL the greatest mortality occurred during the 5th instar in all morphs (ca. 20-35%) (Fig. 1).

Nymphal Developmental Time. The time required by *N. viridula* nymphs to reach adulthood was greater at 15ºC/ 10hL (values ranged for the three types from ca. 63 to ca. 78 days). It was ca. 34-42 days at 22ºC/12hL and ca. 21-

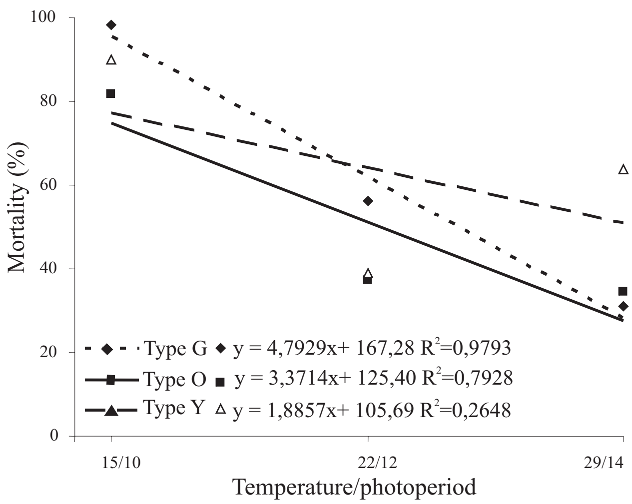

Fig. 1. Percentage mortality of *N. viridula* nymphs of different types under different conditions of temperature/photoperiod (ºC/hL) in the laboratory. G = *smaragdula*; O = *torquata*; and Y = *aurantiaca.*

23 days at 29ºC/14hL (Table 1). At 15ºC/10hL the type *torquata* (O) showed longer developmental time; however, because only one nymph of types *smaragdula* (G) and of *aurantiaca* (Y) reached adulthood, no statistical comparison could be performed. At 22ºC/12hL, type Y took longer time (ca. 42 days) to complete development, than types G and O (ca. 34-35 days). At 29ºC/14hL, type Y took less time (ca. 21 days) to develop, than types G and O (ca. 23 days) (Table 1).

Adult Longevity. Mean total longevity of adult *N. viridula* varied in the different temperatures/photoperiods tested. In general, adults of types *smaragdula* (G) and *torquata* (O) lived significantly longer than those of *aurantiaca* (Y) (Table 2). For example, at 15ºC/10hL, adults of types G and of O lived for over 67 days, while those of type Y had their longevity drastically reduced to ca. 13 days. At 22ºC/12hL, adults of type G lived for ca. 58 days, less than those of type O (ca. 81 days), but, again, both significantly longer than those of type Y (ca. 39 days). At 29ºC/14hL, adults of G and of O lived for ca. 54-58 days, significantly longer than those of type Y that lived for ca. 19 days.

Considering each type separately, adults of the type G showed higher longevity at 15ºC/10hL, whereas adults of the types O and Y showed no directional trends (Table 2).

Adult Reproductive Performance. Fecundity of *N. viridula* types was highly affected by temperature and photoperiod. At 15ºC/10hL, only females of type *torquata* (O) oviposited (51.5 eggs/female); all females of types *smaragdula* (G) and *aurantiaca* (Y) did not lay eggs under these conditions (Table 3). At 22ºC/12hL, 18.2-50.0% of females of all three types reproduced, with a much lower fecundity in the type Y compared to types G and O (34.5 vs. 114.7 and 147.7 eggs per reproductive female, respectively). At 29ºC/14hL, females of the type Y did not reproduce, whereas females of the other two types showed similar fecundities (Table 3).

Discussion

Results of these laboratory studies demonstrate that the different types of *N. viridula* were variably affected by the different combinations of temperature and photoperiod studied. The fact that type *smaragdula* showed greater nymph mortality at 15ºC/10hL than types *torquata* and *aurantiaca* (Fig. 1) demonstrates that the first type is less adapted to the combination of cooler temperature and shorter photoperiod than the last two types. In contrast, type *smaragdula* had the lowest nymph mortality at 29ºC/14hL. Laboratory studies conducted in Japan showed reduced winter survival of type *smaragdula* than other types (R = yellow body with green spots, and F = similar to *torquata* with connexivum yellow), and that type *smaragdula* was more abundant during summer (Kiritani 1970). Additionally, low temperature and short photoperiod is related to the maintenance of the population of type *torquata* during the summer, which contributes to the regulation of *N. viridula* population (Kiritani 1971).

Nymphal developmental time in *N. viridula* is known to be shortened with increase of temperature and lengthening of photoperiod (e.g., Ali & Ewiess 1977, Ali *et al*. 1983, Cividanes & Parra 1994), and the effect of photoperiod on the duration of development is considered to be more

Table 1. Nymphal developmental time (days) of *N. viridula* of different types under variable conditions of temperature and photoperiod in the laboratory [initial number of nymphs]; (number of nymphs).

Types	Temperature/photoperiod		
	15°C/10hL	22°C/12hL	29°C/14hL
smaragdula (G)	67.0 [56] (1)[1]	33.7 ± 0.1 b A [56] (24)	22.8 ± 0.1 a B [58] (40)
torquata (O)	78.3 ± 0.6 A [83] (15)	34.9 ± 0.1 b B [83] (52)	23.5 ± 0.1 a C [76] (50)
aurantiaca (Y)	63.0 [11] (1)[1]	41.7 ± 0.3 a A [36] (22)	20.7 ± 0.7 b B [11] (4)

Means (± SEM) followed by the same lower case letter in each column, and same capital letter in each row do not differ significantly ($P < 0.05$), using the Tukey test.
[1]Data not included in the statistical analysis.

Table 2. Longevity (days) (X ± SEM) of adult (females and males combined) *N. viridula* of different types under variable conditions of temperature and photoperiod in the laboratory (number of adults).

	Temperature/photoperiod		
Types	15/10hL	22/12hL	29/14hL
smaragdula (G)	87.2 ± 7.9 a A (12)	57.7 ± 5.7 b B (12)	57.6 ± 2.7 a B (22)
torquata (O)	67.8 ± 11.5 a AB (6)	81.2 ± 3.0 a A (16)	54.1 ± 2.3 a B (12)
aurantiaca (Y)	13.2 ± 6.2 b B (4)	39.6 ± 4.6 c A (16)	19.5 ± 2.1 b AB (2)

Means (± SEM) followed by the same lower case letter in each column and same capital letter in each row do not differ significantly (P < 0.05) using the Tukey test.

pronounced during the 4th instar (Ali & Ewiess 1977) or during the last two instars as reported in other species of Heteroptera (Musolin & Saulich 1997). More recently, this issue has been revisited by Musolin & Numata (2003a), who analyzed the effects of photoperiod and temperature on three morphs (G, O, and F). In the majority of the areas colonized by *N. viridula*, type G is the most common, which suggests that it is better adapted to different environments (Yukawa & Kiritani 1965, Kiritani 1970). Perhaps, by being less abundant, other types have not been included in studies on the dependence of the duration of nymphal developmental time on temperature and photoperiod. This is an important point that should be considered in future studies. For example, the adaptation of *N. viridula* to new environments and expansion of its distributional range (Musolin & Numata 2003b) might occur with variable intensity if we consider the variation in adaptability of the different types to abiotic conditions. The fact that type *torquata* is better adapted to cooler temperatures might suggest that it will better colonize new areas with colder weather, such as Osaka (Japan) (Musolin & Numata 2003b). In contrast, the expansion of *N. viridula* in the tropics towards warmer areas (Panizzi 2002) would favor types *smaragdula* and *aurantiaca*. Clearly, these considerations need to be investigated.

Development of nymphs and rearing of adults at 15ºC/10hL, that induced only females of types *smaragdula* and *aurantiaca* not to reproduce, is another sign that the type *torquata* performs better in cooler conditions. However, the fact that *smaragdula* adults survived longer under 15ºC/10hL suggests that they may enter reproductive diapause, saving energy for maintenance under these unfavorable conditions; this may make them the most common type during the summer.

The facts that adults of type *aurantiaca* showed reduced longevity in all temperatures, no reproduction at the extremes of temperature/photoperiod used, and low fecundity under 22ºC/12hL compared to the other types, explains at least in part why this golden type is so rare in nature. This genetic quality apparently does not add any significant input to the capacity of *N. viridula* to thrive in conditions other than the favorable ones, as can be derived from the other two types. Therefore, it is not clear how polymorphism added greater adaptive advantage to *N. viridula*, as stated by Kiritani (1970).

This study adds information to the scarce knowledge on the biology of the different types of *N. viridula* in the neotropics, and supports previous findings on the occurrence of type *torquata* restricted to the cooler temperatures of southern Brazil (Vivan & Panizzi in press). Clearly, much remain to be done. For instance, little is known about the differences in feeding preferences among the types except the preliminary report of DeWitt & Armbrust (1978). Moreover, studies to compare the impact of natural enemies (parasites and predators) on the different types of *N. viridula*, and their possible variable susceptibility to the widespread use of pesticides on soybean in this region, await future investigations.

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Table 3. Reproductive performance of females *N. viridula* of different types under different conditions of temperature/photoperiod in the laboratory [number of females]; (number of females that reproduced).

Types	15ºC/10hL			22ºC/12hL			29ºC/14hL		
	% ♀ ovip.	No./♀ Egg masses	No./♀ Eggs	%♀ ovip.	No./♀ Egg masses	No./♀ Eggs	%♀ ovip.	No./♀ Egg masses	No./♀ Eggs
smaragdula (G)	0.0 [12] (0)	-	-	18.2 [12] (2)	2,0 ± 0.0 a	114.7 ± 1.8 ab	66.7 [22] (12)	2.1 ± 0.1a	94.5 ± 0.7 a
torquata (O)	40.0 [6] (2)	1.5 ± 0.4	51.5 ± 1.1	50.0 [16](7)	2.6 ± 0.1a	147.7 ± 5.8 a	45.0 [12] (5)	1.8 ± 0.2 a	93.6 ± 0.6 a
aurantiaca (Y)	0.0 [4] (0)	-	-	37.5 [16] (2)	1.5 ± 0.4 a	34.5 ± 15.2 b	0.0 [2]	-	-

Means (± SEM) followed by the same letter in each column do not differ significantly (P < 0.05) using the Tukey test.

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