ECOLOGY, BEHAVIOR AND BIONOMICS

Effect of Relative Humidity on Emergence and on Dispersal and Regrouping of First Instar *Nezara viridula* (L.) (Hemiptera: Pentatomidae)

Edson Hirose[1], Antônio R. Panizzi[2] and Alexandre J. Cattelan[3]

[1]*Depto. Zoologia, Univ. Federal do Paraná, C. postal 19020, 81531-990, Curitiba PR*
[2]*Lab. Entomologia, Embrapa Soja, C. postal 231, 86001-970, Londrina, PR, panizzi@cnpso.embrapa.br; to whom correspondence should be addressed*
[3]*Lab. Microbiologia, Embrapa Soja, C. postal 231, Londrina 86001-970, PR*

Neotropical Entomology 35(6):757-761 (2006)

Efeito da Umidade Relativa na Eclosão e na Dispersão e Reagrupamento do Primeiro Ínstar de *Nezara viridula* (L.) (Hemiptera: Pentatomidae)

RESUMO - Estudos em laboratório com ninfas de primeiro ínstar do percevejo verde *Nezara viridula* (L.) indicaram que a umidade relativa (UR) afetou significativamente a eclosão e a sobrevivência das ninfas até o segundo ínstar, alcançando o valor máximo (≈ 90%) com a UR > 80%. Com 60% de UR, 60% das ninfas eclodiram e sobreviveram, enquanto com 0% de UR apenas 15% das ninfas eclodiram e a maioria morreu. As ninfas eclodidas de massas de ovos colocadas em caixas de plástico com gradiente de umidade permaneceram sobre os córions (cascas dos ovos) por cerca de um dia. Após esse período elas dispersaram e reagruparam sobre os córions 6.8 ± 0.67 vezes, até o abandono dos córions em direção à fonte de umidade, evitando as áreas saturadas com água. A duração de cada comportamento de rearranjo (dispersão + agrupamento) aumentou com o tempo, variando de ≈ 26 min a 44 min. A duração média em que as ninfas ficaram agrupadas após cada rearranjo decresceu de cerca de 102 min, para o primeiro rearranjo, até 24 min, para o sexto e último rearranjo. Esse comportamento de rearranjo do primeiro ínstar sobre os córions aparentemente compensa a perda de água pelas ninfas.

PALAVRAS-CHAVE: Heteroptera, percevejo, comportamento, agregação, microclima

ABSTRACT - Laboratory studies with 1st instar of southern green stink bug, *Nezara viridula* (L.) indicated that relative humidity (RH) greatly affected nymph emergence and survivorship up to the 2nd instar, reaching the maximum value (≈ 90%) with RH of > 80%. At 60% RH, 60% of the nymphs emerged and survived, while with 0% RH only ≈ 15% of eggs hatched, and most nymphs died. Emerged nymphs from egg masses placed in plastic boxes with a gradient of humidity remained on egg shells for ca. one day. After this period, they dispersed and regrouped on top of shells 6.8 ± 0.67 times, until they abandoned the shells toward the source of humidity, avoiding the water-saturated areas. Duration taken for each rearrangement (dispersal + regroup) increased with time, with a range of ≈ 26 min to 44 min. The mean duration of the grouping behavior on egg shells after each rearrangement decreased from ≈ 102 min (1st) to 24 min (6th and last grouping). The rearrangement behavior of 1st instars on top of egg shells apparently compensates for the water loss of nymphs.

KEY WORDS: Heteroptera, stink bug, behavior, aggregation, microclimate

The southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) is a polyphagous stink bug on several crops of economic importance, and many aspects of its biology, ecology and behavior have been investigated (see Todd 1989, Panizzi *et al.* 2000). Despite this, a detailed study of 1st instar emergence and survivorship during this age until reaching the 2nd instar, and dispersal and aggregation mediated by abiotic factors (e.g., relative humidity) is poorly understood.

N. viridula usually oviposits on the lower (abaxial) surface of leaves, and, after emergence, 1st instars remain aggregated and in close contact with egg shells (Kiritani 1964, Lockwood & Story 1986). Aggregation of 1st instars *N. viridula* is reported to speed development and to reduce mortality (Lockwood & Story 1986), and this behavior is believed to facilitate acquisition of simbionts deposited on the egg's surface by females of heteropterans during oviposition (Abe *et al.* 1995). Other possible function for aggregation during the 1st instar is to avoid desiccation, which is critical at this time.

Relative humidity affects development and survivorship of insects, which must keep body water content within certain limits, and that is influenced by the degree of cuticle permeability (Willmer 1982, Raghu *et al*. 2004). Apparently the ability to maintain body water content during the 1st instar is variable among heteropterans, since early instar aggregation may or may not occur during this stage of development (Panizzi 2004). However, for those species that show gregarism, such as *N. viridula*, in low relative humidity conditions, aggregated nymphs survive better and develop faster than isolated nymphs (Lockwood & Story 1986).

This study was conducted to investigate the impact of relative humidity on *N. viridula* 1st instar emergence and survivorship up to the 2nd instar, on duration of 1st instar dispersal and ability to regroup (on egg shells) after each break up of the cluster, and on the duration of each subsequent grouping (i.e., time nymphs stayed aggregated after each rearrangement), before molting to 2nd instar.

Material and Methods

First instar emergence and survivorship of *N. viridula* under different relative humidity conditions. Egg masses of *N. viridula* were obtained from a laboratory colony established at the Laboratório de Entomologia of Embrapa Soja, in Londrina, PR. On the day of egg deposition, each egg mass was hand removed from soybean [*Glycine max* (L.) Merrill] plants, and they were cemented with starch glue on a plastic stripe (1.0 x 1.5 x 0.1 cm), and put on a plastic holder inside a glass vial (300 ml) with hermetic cap. Six treatments (vials) were prepared containing: 50 ml of distilled water (100% RH), saturated salt solution of KCl (84% RH), NaCl (76% RH), $Mg(NO_3)_2.6H_2O$ (55% RH), $MgCl_2.6H_2O$ (33% RH), and 50 g silica gel (SiO_2) (0% RH). Each treatment was replicated five times (n = 6 treatments x 5 replicates = 30 egg masses). Vials were put at random in an environmental chamber (25 ± 1ºC, 14hL:10hD). Daily observations were made, and the number of emerged nymphs and their survivorship until they reached the 2nd instar was recorded.

Dispersal and rearrangement of first instar *N. viridula* in the presence of water source. Egg masses of *N. viridula* obtained from the laboratory colony were put in closed transparent plastic boxes (arenas - 11.0 x 11.0 x 7.0 cm), partially lined with filter paper (11.0 x 9.7 cm); a strip of filter paper (1.0 x 11.0 cm) saturated with distilled water by capillarity was placed on one end of the arena (Fig. 1). Permanence of 1st instars on the egg shells (time spent stationary) and rearrangement (time spent mobile, i.e., dispersal + regroup) behavior of nymphs after emergence were recorded using a digital photographic camera (Creative NX) assembled over the arena, which was linked to a computer (Fig. 2). Recordings were taken continuously for 72h, and the position of nymphs registered every 5 min. This procedure was repeated five times.

Data analysis. The percentages of nymphs that emerged and survived from eggs that were submitted to different relative humidity were calculated. Mean duration (min) of permanence of 1st instars on the egg shells (time spent stationary), and mean duration of nymphs' rearrangement (time spent mobile), were calculated through the analysis of the images obtained from the digital photographic camera. Data were submitted to analysis of variance (ANOVA), and the means were compared using the Tukey test ($P < 0.05$), calculated with the program Statistica version 6.0 (StatSoft 2001). The percentage of nymphal emergence related to the percentage relative humidity was analyzed using linear regression.

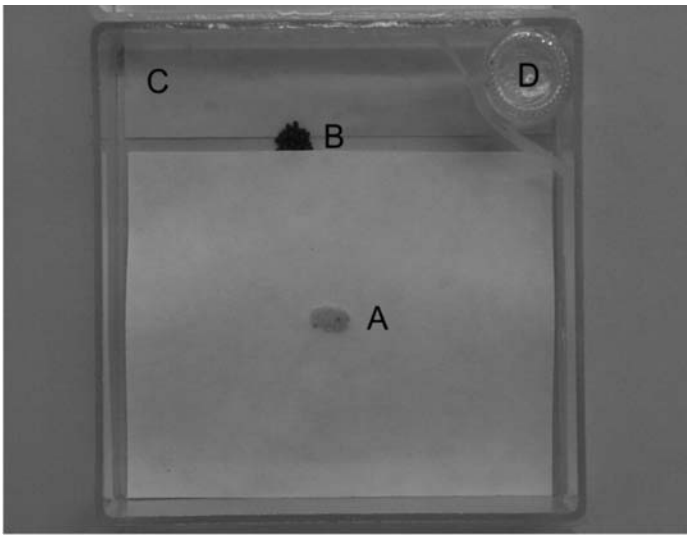

Fig. 1. Transparent plastic box (arena) used to monitor the movement of *N. viridula* 1st instars toward the humidity source. A = egg shells (corions); B = 1st instars grouped by the filter paper saturated with distilled water; C = strip of filter paper saturated with distilled water; and D = source of distilled water.

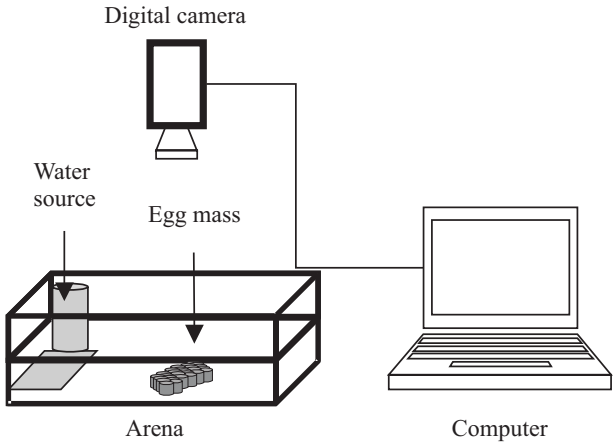

Fig. 2. Schematic representation of the device used to record *N. viridula* nymphs emergence and movement during the 1st instar toward the source of humidity.

Results and Discussion

First instar *N. viridula* nymphal emergence and survivorship up to the 2nd instar was affected by the percentage of relative humidity (Fig. 3). At 0% RH, although ≈ 15% of eggs hatched, most nymphs were unable to emerge, remaining with part of their body in the egg shells. The nymphs that completed emergence, died soon after; this only happened at this RH condition. As RH increased, the percentage of nymphs that emerged and survived also increased, with intermediate values (60% nymph emergence at ≈ 60% RH), reaching the maximum value (≈ 90% emergence) with RH of > 80% (Fig. 3). These data demonstrate that humidity is critical for nymph emergence and survival of *N. viridula*. In the field, *N. viridula* deposits egg masses on the lower surface of leaves (Todd 1989). This behavior may increase humidity around egg masses due to condensation of water improving nymph emergence and survivorship. Low RH is reported to prevent embryo development and nymph emergence of the reduviid *Triatoma brasiliensis* Neiva, due to loss of lubrication and cuticular softness (Guarneri *et al.* 2002). In addition, dense foliage provides shelter to insects that seek shaded and moisten areas of the host plant with increasing temperature and declining relative humidity (Raghu *et al.* 2004). In low RH, 1st instars of several species of pentatomids are reported to drink water, and RH below 20% prevents development and emergence (Javahery 1994).

At emergence, *N. viridula* nymphs are orange colored and after 57.0 ± 4.42 minutes they acquire a brown color. Soon after emergence, and after each molt (teneral period), nymphs are more susceptible to water loss due to greater permeability of the cuticle; it is well known that during sclerotization, a dramatic loss of the water through the cuticle occurs (Hopkins & Kramer 1992). The content of water in insects usually varies from 60% to 80% of body weight. However, water content is affected by the environmental temperature and humidity, the developmental phase and transpiration through body surface (Chapman 1998). Eggs lose water through micropylar processes and the nymphs via body surface, particularly during early instars, because the ratio surface/volume is larger at this phase than it is in adults (Bursell 1974, Hinton 1981).

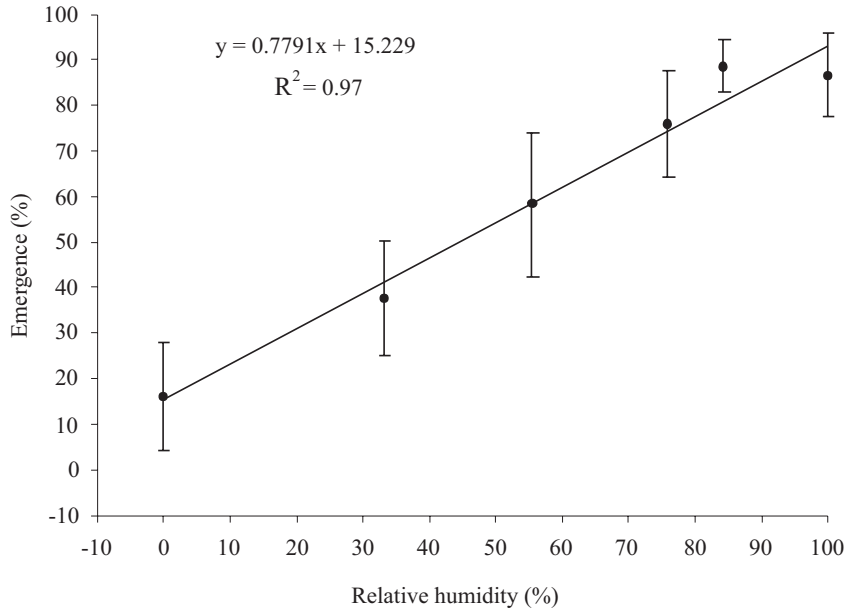

Fig. 3. Linear regression of percentage of the relative humidity and percentage of the emergence of 1st instar *N. viridula*, whose eggs were submitted to different values of relative humidity, using saturated salts solution and silica gel at 25ºC in the laboratory (n = 5).

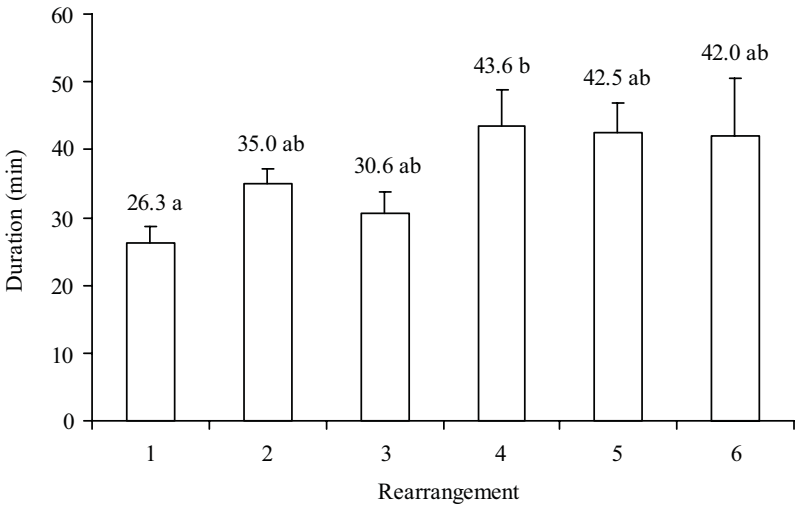

Fig. 4. Mean (± SEM) duration (min) of each rearrangement behavior (time spent mobile) of 1st instar *N. viridula* on egg shells in the laboratory. Means followed by the same letter do not differ significantly ($P < 0.05$) using the Tukey test.

Lockwood & Story (1985) demonstrated that most egg masses of *N. viridula* hatch during a certain period (3:00 to 9:00 AM) under photoperiod of 14 hL:10 hD and temperature of 25ºC and of 28ºC. This suggests that nymphs adjust their emergence to the time when relative humidity is greater, which will increase survival of 1st instars. A way to overcome desiccation at nymph emergence is to emerge at dawn, when RH reaches its daily maximum, as reported to different species of heteropterans (Lazzari 1991, Schilmann 1998, Guarneri *et al.* 2002).

Nymphs that emerged from egg masses placed in the center of arenas with a gradient of RH (Fig. 1A) remained on the egg shells (time spent stationary) for ca. one day (21.7 ± 0.67 h). After this period, they moved around the egg shells temporarily (time spent mobile), and returned to regroup on the top of them, repeating this process 6.8 ± 0.67 times. The purpose of going back to the shells is not clear, and might be related to attraction of nymphs back to the egg residues. Shell abandonment occurred 31.2 ± 1.36 h after emergence, and ca. 9h after the beginning of the rearrangements (time spent mobile).

The duration taken for each rearrangement (time spent mobile) was variable, and tended to increase with time with a range of ≈ 26 min for the first to 44 min for the last rearrangement (Fig. 4). Considering the mean time for the first three rearrangements (30.4 min), a significant difference in time was observed when compared to the last three rearrangements (42.8 min). This means that as 1st instars grew older and bigger, the breakups of the cluster became more frequent, and accommodation of the group in a single unit turned more complicated.

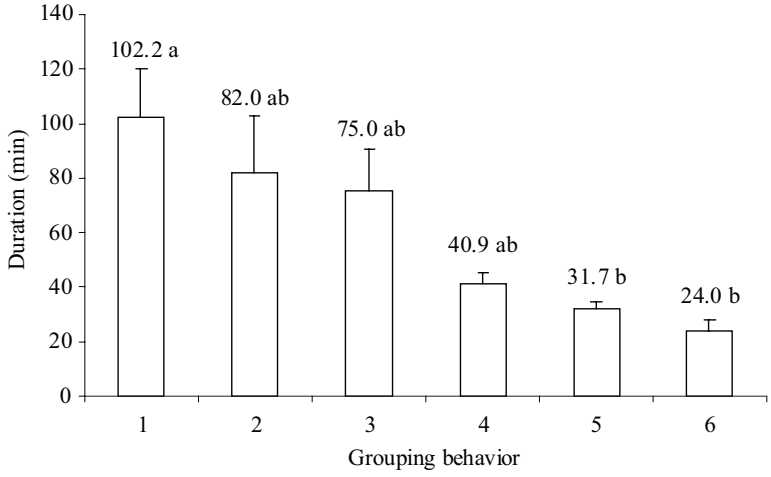

Fig. 5. Mean (± SEM) duration (min) of the grouping behavior (time spent stationary on egg shells in group after each rearrangement) of 1st instar *N. viridula*, in the laboratory. Means followed by the same letter do not differ significantly ($P < 0.05$) using the Tukey test.

The mean duration of the time 1st instar *N. viridula* spent stationary (i.e., stayed in group after each rearrangement on egg shells) decreased with time (Fig. 5). It varied from ≈ 102 min for the 1st grouping to 24 min for the 6th and last grouping. Significant differences, however, were only observed between the first and the last two grouping behavior. Afterwards, they finally abandoned the egg shells. Nymphs moved from the egg shells to areas with higher humidity, first at random, and then forming one or two groups by the strip of paper saturated with distilled water (Fig. 1B). In general, all nymphs joined the group or groups, but in some cases few nymphs remained disperse. It is interesting to note that nymphs avoided staying on the wet area (strip of water-saturated paper - Fig. 1C). Apparently the excess of water is harmful to 1st instars, and in many occasions we have observed nymphs to disperse and die when excess of water occurred in the rearing container (ARP, unpublished). The direct and constant contact with water in the liquid form may cause drowning, and consequently death of nymphs. In laboratory rearing of *N. viridula*, 1st instars, which are known not to feed, move from egg shells and aggregate on pods of green beans (*Phaseolus vulgaris* L.) (Jones 1985), and this is known to increase their survivorship, probably due to the increment of humidity in the environment. First instars *N. viridula* during development are able to increase weight by drinking or acquiring water directly from the atmosphere in high humidity conditions (Lockwood & Story 1986).

In conclusion, these laboratory results demonstrate that *N. viridula* 1st instar are very susceptible to changes in relative humidity, and need high levels of RH to emerge from egg shells and survive during this age. Considering that aggregation should keep humidity in a suitable level, it is reasonable to conclude that the rearrangement behavior on the top of egg shells apparently compensates for the water loss of nymphs. In the presence of a gradient of humidity, nymphs tend to break up the colony with greater intensity and take longer time to regroup as they grew older and seek areas with greater RH, but do not tolerate wet areas that cause massive mortality at this period of development. Finally, this rearrangement behavior may or may not occur in the field, depending on the RH condition. It seems to be relevant when the RH drops, as might happens inside rearing containers in artificial colonies kept in the laboratory.

Ackowledgments

We thank Thomas A. Coudron and Lenita J. Oliveira for critical reading of the manuscript. This research was supported by a scholarship to EH from the National Council for Scientific and Technological Development (CNPq), and through laboratory facilities provided by the Embrapa National Soybean Research Center, both from Brazil.

References

Abe, Y., K. Mihiro & M. Tanakashi 1995. Symbiont of brown-winged green bug, *Plautia stali* Scott. Jpn. J. Appl. Entomol. Zool. 39: 109-115.

Bursell, E. 1974. Environmental aspects – humidity, p.44-84. In M. Rockstein (ed.), The physiology of Insecta. New York, Academic Press, v. 2, 568p.

Chapman, R.F. 1998. The insects: Structure and function, 4th ed., New York, Harvard University Press, 770p.

Guarneri, A.A., C. Lazzari, L. Diotaiuti & M.G. Lorenzo. 2002. The effect of relative humidity on the behavior and development of *Triatoma brasiliensis*. Physiol. Entomol. 27: 142-147.

Hinton, H.E. 1981. Biology of insect eggs, v. 1, New York, Pergamon Press, 473p.

Hopkins, T.L. & K.J. Kramer. 1992. Insect cuticle sclerotization. Annu. Rev. Entomol. 37: 273-302.

Javahery, M. 1994. Development of eggs in some true bugs (Hemiptera-Heteroptera). Part I. Pentatomoidea. Can. Entomol. 126: 401-433.

Jones Jr., W.A. 1985. *Nezara viridula*, p.339-344. In P. Singh & R.F. Moore (eds.), Handbook of insect rearing, v. 1, New York, Elsevier Science, 514p.

Kiritani, K. 1964. The effect of colony size upon the survival of larvae of the southern green stink bug, *Nezara viridula*. Jpn. J. Appl. Entomol. Zool. 8: 45-53.

Lazzari, C.R. 1991. Circadian rhythm of egg hatching in *Triatoma infestans* (Hemiptera: Reduviidae). J. Med. Entomol. 28: 740-741.

Lockwood, J.A. & R.N. Story. 1985. Photic, thermic and sibling influences on the hatching rhythm of the southern green stink bug, *Nezara viridula* (L.). Environ. Entomol. 14: 562-567.

Lockwood, J.A. & R.N. Story. 1986. Adaptive functions of nymphal aggregation in the southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae). Environ. Entomol. 15: 739-749.

Panizzi, A.R. 2004. Adaptive advantages for egg and nymph survivorship by egg deposition in masses or singly in seed-sucking Heteroptera, p.60-73. In G.T. Gujar (ed.), Contemporary trends in insect science, New Delhi, Campus Books International, 424p.

Panizzi, A.R., J.E. McPherson, D.G. James, M. Javahery & R.M. McPherson. 2000. Economic importance of stink bug (Pentatomidae), p.421-474. In C.W. Schaefer & A.R. Panizzi (eds.), Heteroptera of economic importance, Boca Raton, CRC Press, 828p.

Raghu, S., R.A.I. Drew & A.R. Clarke. 2004. Influence of host plant structure and microclimate on the abundance and behavior of a tephritid fly. J. Insect Behav. 17: 179-190.

Schilmann, P.E. 1998. Factores que afectan la reproducción de las vinchucas: Aspectos fisiológicos y comportamentales. PhD thesis, Buenos Aires, University of Buenos Aires, 113p.

StatSoft, Inc. 2001. STATISTICA (data analysis software system), version 6. www.statsoft.com.

Todd, J.W. 1989. Ecology and behavior of *Nezara viridula*. Annu. Rev. Entomol. 34: 273-292.

Willmer, P.G. 1982. Microclimate and the environmental physiology of insects. Adv. Insect Physiol. 16: 1-57.

Received 13/XII/05. Accepted 20/VI/06.