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ORIGINAL ARTICLES

Influence of three constant temperatures on some biological aspects of *Nezara viridula* (L.) and the egg parasitoid, *Trissolcus basalis* (Woll.)

Mohamed A. Khattab

Piercing and Sucking Insect Department, Plant Protection Research Institute, Agricultural Research Center, Egypt.

ABSTRACT

The biological aspects of *Nezara viridula* (L.) as well as the rate of egg parasitism and emergence of parasitoid, *Trissolcus basalis* (Woll.) were studied under three constant temperatures; 20 , 25 and 30 ºC. The egg incubation period of *N. viridula*, duration of nymphal stage and oviposition period decreased with an increase of temperature. The highst rate of nymphs mortality was recorded at the lowest temperature, while the lowest rate occurred at 25 ºC. The adult longevity increased with the increase in temperature and the females lived longer than males for all the considered temperatures. At 20 ºC, the emerging females failed to copulate , consequently they failed to oviposit. The fecundity of females was significantly higher at 30 ºC than at 25 ºC. At 20 ºC, the life span of *N. viridula* was longer for females than for males. The generation period did not significantly differ at 25 ºC and 30 ºC, while the insect did not complete its life cycle at 20 ºC. The parasitism rate of *N. viridula* eggs by *T. basalis* and the parasitoid emergence were higher when females of the parasitoid were kept at 25 ºC, while the lower rats were found at 20 ºC and 30 ºC. Also, the highest period of parasitoid life cycle was recorded at 20 ºC, while the shortest was found at 30 ºC .

Kew words: *Nezara viridula* (L.) , egg parasitoid, *Trissolcus basalis* (Woll.), egg incubation.

Introduction

The green stink bug, *Nezara viridula* (L.) is one of the major insect pests attacking various plant species in different parts of the world (Panizzi *et al*., 2000). In Egypt, recently, its population density has tended to be high causing considerable damage to many field and horticultural crops (Khattab, 2003 and Khalafalla *et al*., 2005a). The scelionid egg parasitoid, *Trissolcus basalis* (Wollaston) is the most important natural enemy of *N.viridula* eggs, as it plays an important role in the regulation of the insect pest population. However, egg parasitism of *N.viridula* is the most effective mortality factor in most affected regions of the world (Awadalla and Shanab, 1993; Awadalla, 1996; Khalafalla *et al*., 2005 b; VanLenteren and Bueno, 2003; Canton-Ramos and Callejon-Ferre, 2010; Wright and Diez, 2011 and Liljesthron *et al*., 2013).

The temperature is one of abiotic factors influencing the population dynamics of insect pests and their natural enemies because of being a very component of the environment of poikilothermic organisms, consequently it has a strong effect on survival, development as well as the duration and number of generations. Ali *et al*., (1979) attributed low summer densities of *N.viridula* to nymphal mortality from exposure to high temperatures (25-28° C). Also, a low rate of reproduction could result from delayed egg maturation, decreased egg laying or lack of suitable host plants. Velasco (1989) reported that mid-summer nymphal numbers were low when mid-summer temperature average about 25°C (max. 32.5°C and min. 18°C) and sometimes reach as high as 38°C. Velasco and Walter (1993), in laboratory studies, showed that high temperatures (27/37°C) adversely affected the nymphal survival and adult reproduction of *N. viridula* compared with lower temperatures (10/20°C and 20/30°C). Clarke and Walter (1993) found that 90% of *T. basalis* population exposed to summer temperatures (mean high temperature > 25°C) died by 55 days, with 80% of mortality occurring in the first 30 days. Panizzi and Niva (1994) reported that when food plants are not available and the abiotic factors as temperature and photoperiod become unfavorable, *N. viridula* will show different overwintering strategies. The insect used winter plant species as overwintering sites because the adults were only moving and feeding on these winter hosts without egg depositing (Khalafalla *et al*., 2005a). Musolin and Numata (2003) mentioned that at 20°C, the development period of *N. viridula* nymphs was significantly shorter under LD 10: 14h. (short day) and LD 16: 8 h (long day) than under intermediate photoperiods, whereas at 25°C, it was slightly shorter under intermediate than short- and long- day conditions.

Corresponding Author: Mohamed A. Khattab, Piercing and Sucking Insect Department, Plant Protection Research Institute, Agricultural Research Center, Egypt.
E-mail: mhmdkhattab@yahoo.com

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Therefore, this work was conducted to study the influence of three constant temperatures on the biological aspects of *Nezara viridula* (L.) as well as on the rate of egg parasitism and emergence of parasitoid, *Trissolcus basalis* (Woll.).

Materials and Methods

1-Culture of Nezara viridula (L.):

The adult green stink bug, *N.viridula* were collected from soybean cultivated during June at Sakha Agriculture Research Station Farm-Kafr EL-Sheikh and introduced into wooden breeding cages (30x30x40cm) with wire gauze sides .Twenty pairs of *N.viridula* adults were placed in each cage and provided with fresh green pods of bean *Phaseolus vulgaris* (L) , which were renewed every two days. The deposited egg –masses were collected and placed in Petri-dishes where introduced into incubators at three different temperatures adjusted previously 20±1, 25±1 and 30±1°C till hatching. The incubation period and hatchability were recorded. For each constant temperature , one hundred newly hatched nymphs were divided into four groups (25nymphs each)and kept in the breeding containers (3L capacity).The insects were fed on the fresh pods of bean and renewed every two days. The duration of the nymphal instars, mortality rate, adult longevity, fecundity, total life span &generation period were recorded.

2-Culture of Trissolus basalis (Woll.):

The secelionid egg parasitoid**,** *T.basalis* was obtained by collecting parasitized *N. viridula* egg –masses from soybean field, which were grown in the same above mentioned Farm. The obtained parasitized egg-masses were kept under laboratory conditions in Petri-dishes containing pieces of moistened cotton wool until hatching to *N. viridula* nymphs and / or parasitoids. The emerging parasitoids were provided with cotton soaked with 10% sugar solution to serve a food source. About 600 fresh egg of *N.viridula* were divided into four groups, 150 eggs for every tested temperature. Every group was kept in cages and exposed to one parasitoid female for 24 hours. Then the parasitoid females were removed and the eggs were held in glass in the incubator until adult emergence. The percentages of egg parasitism and adult emergence and developmental time were calculated. Also, percentage data were subjected to angular transformation before statistical analysis.

The obtained results were statistically analysed using least significant difference to compare differences among means.

Results and Discussions

Biological aspects of the green stink bug, *Nezara viridula* (L.) as well as the rate of egg parasitism and emergence of parasitoid, *Trissolcus basalis* (Woll.) were studied under three constant temperatures; 20, 25 and 30°C.

1-Egg incubation period of N.viridula and hatchability:

The results presented in Table (1) revealed significantly the inverse effect of the temperature on the incubation period of *N.viridula* eggs. The longest incubation period (8.5days) was recorded at 20°C. At 25 and 30°C, the egg incubation periods were 6.5 and 5.5 days, respectively, without significant difference. The egg hatchability significantly recorded the highest rate of 98.5% at 25°C, followed by that at 30°C, (92.5%), while the lowest rate (61.5%) occurred at 20°C.

Table 1: The egg incubation period of *Nezara viridula* (L.) and hatchability at three constant temperatures.

Temperature (°C)	Egg incubation period (day) ± SE*	Hatchability (%)
20 ±1° C	8.5 ±0.29 a	61.5 c
25 ±1° C	6.5 ±0.15 b	98.5 a
30 ±1° C	5.5 ±0.25 b	92.5 b
LSD (5%)	1.599	4.020

SE*= Standard error; Means followed by the same letter, in the column, did not differ according to the least significant difference (LSD) at 5%.

The obtained results agree with those of Abd El-Wahed (1977) who found that egg incubation period of *N.viridula* varied considerably throughout the season and the hatching never occurred during winter.

2-Nymphal stage duration and rate of mortality:

During the nymphal stage, *N.viridula* molted four times, passing through five nymphal instars before adults. At all temperature, the first nymphal instar (Table 2) had the shortest duration, while the fifth instar passed the longest period. At 30°C, the total nymphal stage duration was 25.0±0.87 days and increased with the decrease in the temperature as it reached 39.5± 1.18 days at 20°C. Also, the results in Table (3) showed that of 25°C proved to be the most favorable temperature for the nymphal stage development, as it significantly induced the least mortality rate (13%), while the highest rate of nymphs mortality (78%) was recorded at the lowest temperature (20±1°C).

Table 2: Effects of three constant temperatures on the nymphal stage duration of *Nezara viridula* (L.).

Nymphal instar	20+1° C	25+1°C	30+1°C
	Duration period (day)±SE*	Duration period (day) ±SE*	Duration period (day) ±SE*
1st	5.75±0.41	3.50±0.42	3.25+0.10
2nd	8.25±0.25	4.75±0.15	4.75+0.33
3rd	7.25±0.41	8.00±0.27	5.00+0.05
4th	7.00±0.29	5.50±0.40	5.50+0.22
5th	11.25±0.64	8.50±0.70	6.50 +0.12
Total duration of nymphal stage	39.5±1.18 a	30.25±0.96 b	25.00±0.87 c
LSD(5%)	4.820		

SE*= Standard error.

The obtained results confirmed the findings of Velasco and Walter (1993) who reported that the high temperature adversely affected the nymphal and adult performance of *N. viridula* compared with lower temperatures (10/20 and 20/30° C) as the development duration of nymphs was longer at the low temperature regime (10/20°C) than at the high temperature regime (27/37°C). In contrast, they showed that low nymphal survival to the adult stage under the high temperature regime .Also, Ali *et al* (1983) reported that nymphs of *N.viridula*, reared on 20 °C, recorded the highest mortality rate. Ali *et al*. (1979) attributed low summer density to nymphal mortality from exposure to high temperatures (25-28°C). Velasco (1989) reported that mid- summer nymphal numbers were low when the temperature averaged about 25° C(max .32.5° C and min. 18°C)and sometimes reached as high as 38° C.

Table 3: Mortality percentage during the five nymphal instars of *Nezara viridula* (L.) under three constant temperatures.

Nymphal instar	Mortality % at the three temperatures		
	20±1° C	25±1° C	30±1° C
1st	42	0	0
2nd	2	0	2
3rd	1	0	3
4th	6	3	5
5th	27	10	13
Total	78 a	13 c	23 b
LSD(5%)	4.769		

Means followed by the same letter, in the column, did not differ according to the least significant difference (LSD) at 5%.

3- Adult longevity and fecundity:

The results in Table (4) show that the newly emerged adults did not copulate at 20^{0}C and consequently the females failed to oviposit. However, at 25 and 30^{0}C, the emerging adults successfully mated and started to oviposit after 20.75 and 25.0 days of emergence, respectively. Also, it was clear that oviposition and post-oviposition periods did not significantly differ at 25°Cand 30°C. . The oviposition period ranged from 18.25 days at 25 °C to 18.5 days at 30^{0}C., while the post-ovipositon period varied form 12.75 days at 25 °C to 11.50 days at 30 °C. The results also showed that the females lived longer than males for all the examined temperatures. However, both female and male lived longer at 30°C, while the lowest period of adult longevity was recorded at 20°C. The mean number of eggs deposited by one female in its whole life (fecundity) was significantly higher at 30 ^{0}C than at 25^{0}C, as it was 268.50 and 224.25 eggs, respectively.

Similar results were obtained by Ali *et al.* (1983), who found that duration of the different developmental stages of *N. viridula* ,ovipositon period and longevity of adults were shorter at 30°C than at 20°C, and 25°C proved to be the most favorable temperature .Also, Musolin and Saulich (1997) reported that *N. viridula* growth delayed under short day conditions.

Table 4: Duration of oviposition period, fecundity and longevity of adults *Nezara viridula* (L.) under three constant temperatures.

Temperature °C	Adult longevity (day) ±SE*		Mean duration period (day) ±SE*			No. of eggs /female/ ±SE*
	Female	Male	Pre-oviposition	Ovipositon	Post-oviposition	
20±1°C	38±2.23 b	28.15±2.75c	-	-	-	-
25±1° C	51.75±2.15a	43.15±3.15b	20.75±0.95b	18.15±0.80a	12.75±0.30a	224.25±10.07b
30±1° C	55.00±2.80a	48.5±2.40a	25.00±1.20a	18.5±0.60a	11.5±0.63 a	268.50±12.73a
LSD (5%)	3.722	4.215	2.890	2.421	4.564	14.506

SE*= Standard error; Means followed by the same letter, in the column, did not differ according to the least significant difference (LSD) at 5%.

4-Total life span and generation period:

In general, the life span of *N. viridula* was longer for females than that for males (Table 5). The life spam averaged 86±3.25;88.5±3.75 and 85.5±3.42 days for the female at 20; 25 and 30°C, respectively , while it was 76.15±2.95; 80.00±3.15 and 79.00±3.06 days for the male , respectively.

The generation period lasted 57.5and 55.5 days at 25 and 30°C, respectively. At 20°C, the insect did not complete its life cycle, as the emerging adults failed to copulate, consequently they did not deposit any eggs. The results agree with those of Nilakhe(1976), Jones and Sullivon (1981) and Panizzi and Niva (1994).who reported that, when food plants are not available and abiotic factors as temperature and photoperiod become unfavorable ,*N. viridula* will show different overwintering strategies. Also, Khalafalla *et al*. (2005a) found that *N.viridula* adults were observed to move and feed on winter hosts, but they do not reproduce, as no egg –masses were detectable.

Table 5: Life span and generation period of *Nezara viridula* (L.) at three constant temperatures.

Temperature (°C)	Life span (day) ±SE*		Generation period (day) ±SE*
	Female	Male	
20±1°C	86.0±3.25 a	76.15±2.95 a	---
25±1°C	88.5±3.75 a	80.00±3.15 ab	57.5±2.96 a
30±1°C	85.5±3.42 a	79.00±3.06 b	55.5±2.45 a
LSD (5%)	4.769	2.858	2.233

SE*= Standard error; Means followed by the same letter, in the column, did not differ according to the least significant difference (LSD) at 5%.

5-Parasitoid of Nezara viridula (L.) eggs; Trissolcus basalis (Woll.):

The parasitoid females of *T.basalis* lay their eggs singly in the host. The resultus in Table (6) show that the highest number of parasitized host eggs was recorded when females of the parasitoid were kept at 25°C, while, the lowest number was found at 20°C and 30 °C without significant difference between them.

There was a significant difference in the percentage of emerging parasitoids, as the highest rate occurred at 25°C, while the lowest rate (78%) was found at 20°C compared to those at 20 and 30 °C .Also, the corresponding number of emerging parasitoids was higher at 25°C . Thus, the successful percentage of parasitism was 96% at 25°C, while it was 78%at 20°C .The highest period of life cycle of parasitoid (19days) was recorded at 20°C, while the shortest period (10days) was found at 30 °C.

Table 6: Effects of Three constant temperatures on parasitism and emergence of parasitoid, *Trissolcus basalis* on *Nezara viridula* eggs.

Temperature (C°)	No. of exposed eggs	No. of parasitized host eggs±SE*	% of parasitism	No.of emerging parasitoids±SE*	Successful parasitism %	Life cycle (day) ±SE*
20±1°C	150	120.0±2.34 b	80	117.0±2.64 b	78	19.0±0.82 a
25±1° C	150	145.5±2.82 a	97	144.0±2.70 a	96	12.0±0.82 b
30±1° C	150	127.5±2.34 b	85	124.5±3.60 b	83	10.0±1.41 b
LSD (5%)	__	13.538	__	14.395	__	2.158

SE* = Standard error; Means followed by the same letter, in the column, did not differ according to the least significant difference (LSD) at 5%.

These results are in agreement with those of Jones *et al*. (1996) who reported that the parasitism on *N. viridula* eggs ranged from 11.1% to 50%. Shepard *et al*. (1994) mentioned that the percentage of parasitism of *N.viridula* eggs in the field was variable among years and crops. Also, Awadalla (1996) reported that the rate of parasitism of *N. viridula* eggs by *Trissolcus megallocephalus* (Ashmead) and the number of emerged parasitoid increased when the temperature increased from 20°C to 25°C. Khalafalla *et al*., (2005b) found that the parasitism of *N.viridula* eggs and the parasitoids emergence were high during June, July and August and this was synchronized with the increase of temperature at these months.

References

Abd El-Wahed, M.S., 1977. Biology and ecology of the green cotton bug, *Nezara viridula* L. in Egypt (Hemiptera-Heteroptera, Pentatomidae). M.Sc. Thesis, Fac. of Agric., Ain Shams Univ., 96 pp.

Ali, M.A., A.M. Awadallah and A.A. El-Rahman, 1979. A study on the phenology and ecology of the green stink bug *Nezara viridula* L. (Heteroptera: Pentatomidae). Zeitschrift Fur Angewandt Ent., 88(5): 476-483.

Ali, M.A., A.M. Awadallah and A.A. El-Rahman, 1983. Studies on temperature and food plants as ecological factors influencing the bionomics of the green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). Agric. Res. Rev., 61(1): 165-177.

Awadalla, S.S., 1996. Influence of temperature and age of *Nezara viridula* L. eggs on the Scelionid parasitoid, *Trissolcus megallocephalus* (Ashm.) (Hym., scelionidae). J. Appl. Ent., 120(7): 445-448.

Awadalla, S.S. and L.M. Shanab, 1993. Parasitoid-host relationship between the parasitoid *Trissolcus* sp. (*Microphanarus megallocephalus* Ashm.) and its host, *Nezara viridula* L. J. Agric. Sci. Mansoura Univ., 18(8): 2441-2446.

Canton-Ramos, J.M. and A.J. Callejon-Ferre, 2010. Raising *Trissolcus basalis* for the biological control of *Nezara viridula* in greenhouses of Almeria (Spain). African J. Agric. Res., 5(23): 3207-3212.

Clarke, A.R. and G.H. Walter, 1993. Biological control of green vegetable bug *Nezara viridula* (L.) in Eastern Australia: Current Status and prospective, pp. 223-225. In: Corey, S.A.; Dall, D.J. and Milne, W.M. [Eds.], Pest Control and Sustainable Agriculture, CSIRO, Canberra, Australia.

Jones, W.A. and M.J. Sullivan, 1981. Overwintering habitats, spring emergence patterns, and winter mortality of some South Carolina Hemiptera, Environ. Ent., 10: 409-414.

Jones, W.A., B.M. Shepard and M.J. Sullivan, 1996. Incidence of parasitism of pentatomid (Heteroptera) Pests of soybean in South Carolina with a review of studies in others states. J. Agric. Ent., 13(3): 243-263.

Khalafalla, E.M.E., Z. Shenishen, I.S. El-Hawary and M.A. Khattab, 2005a. The host range and overwintering sites of *Nezara viridula* L. under Kafr El- Sheikh conditions. Egypt. J. Agric. Res., 83(1): 79-85.

Khalafalla, E.M.E., R.E. El-Sufty, I.S. El-Hawary and M.A. Khattab, 2005b. Parasitism of *Nezara viridula* L. eggs under field conditions at Kafr El- Sheikh Governorate. Egypt. J. Agric. Res., 83(1): 87-94.

Khattab, M.A.A., 2003. Ecological and biological studies on the green bug, *Nezara viridula* L. (Pentatomidae: Hemiptera). Ph.D. Thesis, Fac. Agric. Tanta Univ., pp: 147.

Liljesthrom, G.G., M.F. Cingolani and J.E. Rabinovich, 2013. The functional and numerical response of *Trissolcus basalis* (Hymenoptera: Platygastridae) parasitizing *Nezara viridula* (Hemiptera: Pentatomidae) egg in the field .Bull. Ent. Res., 18: 1-10.

Musolin, D.L. and H. Numata, 2003. Photoperiodic and temperature control of diapauses induction and control change in the southern green stink bug *Nezara viridula*. Physiological Ent., 28(2): 65-74.

Musolin, D.L. and A.K. Saulich, 1997. Photoperiodic control of nymphal growth in true bugs (Heteroptera). Ent. Rev., 77(6): 768-780.

Nilakhe, S.S., 1976. Overwintering, survival, fecundity and mating behaviour of the rice stink bug. Ann. Ent. Soci. Am., 69: 717-720.

Panizzi, A.R. and C.C. Niva, 1994. Overwintering strategy of the brown stink bug in northern Parana. Pesq. Agropec. Bras., 29: 509-511.

Panizzi, A.R., J.E. McPherson, Dd.G., Dd.g. James, M. Jav.ahery and R.M. McPherson, 2000. Stink bugs (Pentatomidae), pp 421. In C.W. Schaefer, and A.R. Panizzi (eds.),Heteroptera of Economic Imporatance. CRC, Boca Raton, FL, 828pp.

Shepard, B.M., K.D. Elsey, A.E. Muckenfuss and H.D. Justo, 1994. Parasitism and predation on egg masses of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae), in tomato, okra, cowpea, soybean, and wild radish. J. Agric. Ent., 11(4): 375-381.

VanLenteren, J.C. and V.H. Bueno, 2003. Augmentative biological control of arthropods in Latin America. Bio. Control, 48: 123-139.

Velasco, L.R.I., 1989. Influnce of host plants in the population dynamics of the green vegetable bug *Nezara viridula* (Linn.) in SE Queensland, Australia. Ph.D. Thesis, Queensland Univ.

Velasco, L.R.I. and G.H. Walter, 1993. Influnce of temperature on survival and reproduction of . *Nezara viridula* (L.) (Hemiptera: Pentatomidae). J. Aust. Ent. Soc., 32: 220-228.

Wright, M.G. and J.M. Diez, 2011. Egg parasitism by *Trissolcus basalis* (Hymenoptera: Scelionidae) in architecturally varied habitats, and observations on parasitism in Macadamia nut orchards and other habitats following Augmentative release. Proc. of the Hawaiian Ent. Soc., 43: 23-31.