

INFLUENCE OF TEMPERATURE AND HUMIDITY REGIMES ON THE DEVELOPMENTAL STAGES OF GREEN VEGETABLE BUG, *NEZARA VIRIDULA* (L.) (HEMIPTERA: PENTATOMIDAE) FROM INLAND AND COASTAL POPULATIONS IN AUSTRALIA

Pol Chanthy^{1*}, Robert J. Martin^{2^}, Robin V. Gunning³ and Nigel R. Andrew¹

¹ Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale NSW 2351, Australia

² Agricultural Systems Research Cambodia Co. Ltd., Battambang 02353, Cambodia

³ Tamworth Agricultural Institute, NSW Department of Primary Industries, 4 Marsden Park Road, Calala, NSW 2340, Australia

* Cambodian Agricultural Research and Development Institute (CARDI), P.O. Box 1, Phnom Penh 12302, Cambodia

^ Maddox Jolie-Pitt Foundation (MJP), Group #02, Rumchek4 village, Rotanak Commune, Battambang 02353, Cambodia
Email: chanthypol@gmail.com

Summary

Laboratory studies were conducted to assess impacts of temperature and humidity regimes on the development of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) from inland and coastal populations in NSW, Australia. Four temperature regimes, 25±2°C, 30±2°C, 33±2°C, and 36±2°C and two humidity regimes, 40±10% and 80±10% RH were applied in the experiment with a constant photoperiod of 14:10 h (L:D). The developmental time of the nymphal stage of *N. viridula* significantly decreased with increasing temperature. Percentage nymphal survival significantly decreased with increasing temperature or high humidity (80% RH) regimes. Longevity of *N. viridula* adults declined with increasing temperature or high humidity regimes and female longevity was longer than males. High temperatures (30, 33 and 36°C) or high humidity significantly reduced reproductive performance and capacity of *N. viridula* compared to low temperature (25°C) or low humidity (40% RH). However, high humidity significantly increased egg hatchability of *N. viridula* compared with a low humidity regime. Interactions of temperature and humidity regimes significantly changed incubation period, adult longevity, mating frequency, pre-mating period, egg-mass size and egg hatchability of *N. viridula*. Interactions of population location (coastal or inland), temperature and humidity regimes significantly changed incubation period and pre-oviposition period of *N. viridula*. Temperature and humidity are important environmental factors for the development and reproduction of *N. viridula*. Higher temperatures shorten the length of nymphal duration, but reduce nymphal survival. The optimum temperature for the development and reproduction of *N. viridula* was 25°C with 40 ± 10% RH. No differences in nymphal duration, nymphal survival, adult longevity or reproduction performance between inland and coastal *N. viridula* populations were found under different climate conditions. We show the importance of assessing all life-stages in the response to varying temperature and humidity regimes, especially in terms of assessing responses to climate change.

Key words: Pentatomidae; climate change; nymph, adult; life history; insect; agriculture.

INTRODUCTION

Green vegetable bugs, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) are a common polyphagous insect (Ali and Ewiess 1977). Kamal (1937) cited in Ali and Ewiess (1977) first reported that *N. viridula* was primarily known as a pest of cotton in Egypt. This pest is widely distributed in different parts of the world and is a serious pest of a range of economic crops (Ali and Ewiess, 1977, Kamal 1937). The range of geographical distribution of *N. viridula* extends across temperate and tropical areas, including the Americas, Africa, Asia, Australia and Europe (Ali and Ewiess 1977, Singh and van Emden 1979, Todd 1989, Waterhouse 1998). *N. viridula* is a highly polyphagous pest attacking both monocots and dicots, but it appears to have a preference for leguminous plants (Todd 1989).

In Australia, *N. viridula* is a pest of many horticultural and field crops. *N. viridula* became established in the Sydney area as early as 1911, and by 1938 was recorded in all mainland states (Clarke 1992). *N. viridula* is a major pest of soybean in

southern Queensland (Evans 1985, Turner and Titmarsh 1979) and northern New South Wales (NSW) (Brier and Rogers 1991, Miller *et al.* 1977). However, it is not restricted to that crop and occurs regularly on other hosts including sorghum, wild crucifer, lucerne, sunflower, castor bean and corn (Knight and Gurr 2007, Velasco and Walter 1992).

As for other pest species, and insects in general (Andrew 2013, Andrew and Terblanche 2013), the distribution, abundance and management of *N. viridula*, is likely to be affected by climate change. It is likely that pests, particularly those of tropical or semi-tropical origin, could spread southward in Australia, or contract from cropping regions affected by reduced rainfall and rising temperatures (Pittock 2003). For insects, such as GVB, different parts of its current regional distribution will be exposed to different impacts of climate change into the future (Andrew *et al.* 2013, Andrew and Terblanche 2013). In the inland North West region of NSW including the Breeza area, the climate is predicted to be hotter in all seasons by 2050, with the greatest warming in

spring and winter (NSW Climate Impact Profile 2010). Average daily maximum and minimum temperatures are very likely to increase by between 1 and 3°C in different parts of the region. Rainfall is likely to increase in all seasons, except winter. On the other hand, the north coastal region of northern NSW average daily maximum temperature is predicted to increase in all seasons by 2050. The smallest increases are projected to occur in summer (1.0-1.5°C) and the greatest in winter (2.0-3.0°C). Average daily minimum temperature is projected to increase by 2.0-3.0°C in all seasons. Spring rainfall is not expected to change. Summer and autumn rainfall is expected to increase slightly, while winter rainfall is expected to decrease slightly (NSW Climate Impact Profile 2010).

Such increases in temperature could influence insect herbivore populations in various complex ways (Hughes 2000, Yamamura and Kiritani 1998). The effect of climate change on insect herbivores can be direct, through impacts on insect phenology, life cycles, and distribution (including movement and migration), or indirect where the insects respond to climate-induced changes mediated through other factors, especially the host plant (Bale *et al.* 2002). As with other insect species, terrestrial and aquatic Heteroptera species respond to climate change by shifting their distribution ranges, changing abundance, phenology, voltinism, physiology, behaviour, and community structure (Andrew *et al.* 2013, Musolin 2007). Many species of true bugs (Heteroptera) have been recently reported to change their distribution ranges, presumably in response to climate change (Musolin and Fujisaki 2006). Such expansion of ranges of individual species can enrich local faunas and change community structure, especially at the northern latitudes. The ongoing warming is expected to further affect the ecology and distribution of true bugs (Musolin and Fujisaki 2006).

The effects of temperature increases on insects are likely to be pronounced in most, if not all, regions globally. The interaction between temperature and other factors such as rainfall may become important in tropical regions (Bale *et al.* 2002). Cammell and Knight (1992) stated that the evaluation of any changes in insect herbivore populations should be studied within these contexts. Interaction between the different climate change factors needs considerably more detailed investigation. The direct effect of temperature may be modified by increased precipitation, for instance, precipitation inland and coastal northern NSW is expected to increase from 5-20% in spring, summer and autumn, but to decrease

in winter from 10-20% for Breeza (inland) and 5-10% for Grafton (coastal) by 2050 (NSW Climate Impact Profile 2010). This, in turn, is likely to affect relative humidity, which is important for many physiological functions, such as reproduction. Moreover, direct effects of climate change on insect performance need to be set in a wider context and attention given to how the direct effect of temperature will interact with other factors, particularly natural enemies and host plant conditions (Andrew *et al.* 2013, Bale *et al.* 2002).

In this study we assessed the impacts of predicted climate change scenarios on development of *N. viridula*. The impact of low and moderate temperature regimes on *N. viridula* survival and development has previously been studied (Ali and Ewiess 1977, Harris and Todd 1980) as have the effects of relatively high temperature fluctuations (Velasco and Walter 1993), who examined the influence of fluctuating temperatures of 10-20, 20-30 and 27-37°C on nymphal survival and developmental rates (eggs to adult), and the reproductive capacity of resulting adults. They found that at high fluctuating temperature of 27-37°C adversely affected nymphal survival and adult reproduction compared to lower temperature regimes of 10-20°C and 20-30°C. However, very little is currently known of the interactions of temperature and humidity on insect development, which severely inhibits our ability to make critical predications about insect responses to climate change (Durak and Borowiak-Sobkowiak 2013, Radchuk *et al.* 2013). This study examines the effects of the combination of three factors: location, temperature and humidity regimes on the life cycle of *N. viridula* (eggs to adult). We then assess survival rates of nymphs relative to adults, and reproductive capacity (egg-masses per female, size of egg-mass and fecundity), and egg hatchedability under the different temperature and humidity regimes in the laboratory.

MATERIALS AND METHODS

Collection and rearing

Adults of *N. viridula* (L.) were collected between March and May 2010 in soybean fields at climatically different locations: Breeza (31°14'54"N 150°28'02"E) located in inland North-Western NSW and Grafton (29°41'34"N 152°55'56"E) located on the North Coast of NSW. Inland GVB populations were collected from Breeza, which represents a dry climate (621 mm annual average rainfall) compared to coastal populations collected from Grafton, 1073 mm). The coastal environment is also more humid with average annual 3pm relative humidity of 53% compared to

46% at inland. Average annual maximum temperature is similar at both sites (26°C) but minimum temperature is higher near the coast (Grafton, 13.7°C) compared to inland (Breeza, 10.9°C) (BoM 2011). The study was carried out on private lands and the owners of these properties gave permission to conduct the study here.

Nezara viridula were collected from soybean fields by sweep net or beat sheet and placed into plastic containers (64 mm deep and 118 mm in diameter) (Chantry *et al.* 2013). Air supply was provided by cutting a small hole (65 mm of diameter) in the lid and fitted with mosquito netting. The bugs were provided fresh green bean pods (*Phaseolus vulgaris*) plus water in a cotton wick as food. All the samples were brought to the laboratory for culture. To obtain egg-masses of *N. viridula*, several pairs (30-40 pairs) were reared in a rearing cage (Bug Dorm-4030[®], 30 cm x 30 cm x 30 cm) and maintained in a culture room at 25 ± 2°C under photoperiodic conditions of light:dark (L:D) 14:10 h and 60 ± 10% relative humidity. Egg-masses of *N. viridula* were collected and placed on the lid of small containers (30 ml) near a piece of distilled water soaked cotton wick. Egg-masses were then placed into large containers (64 mm deep and 118 mm in diameter) with air supply provided through holes in the lids fitted with nylon mesh until nymphs hatched. Most of the first instar nymphs moulted to second instar nymphs 4 or 5 days after hatching under this culture room condition without food. New second instar nymphs were provided with fresh green beans as food. Rearing and experiments were conducted in the Insect Ecology Laboratory, Zoology, University of New England, Australia.

Rearing experiment

The experiment was laid out in a three way factorial design (n =3). Factors were temperature, humidity, and location with three replications. Four temperature regimes (25 ± 2°C, 30 ± 2°C, 33 ± 2°C and 36 ± 2°C) and two humidity regimes, 40 ± 10% and 80 ± 10% were applied in the experiment. Temperature was considered as the main factor. There were 16 treatments (4 temperature regimes x 2 humidity regimes x 2 locations with 3 replicates of each). The experiment was conducted under consistent photoperiod conditions (L:D) 14:10 h. Saturated salt solutions were used to maintain humidity regimes in incubators. Saturated lithium chloride (LiClH₂O) plus distilled water (4:1) was used to keep the humidity regime in incubators at 40% RH and saturated magnesium chloride (MgCl₂.6H₂O) plus distilled water (1:1) was used to create 80% RH (Winston and

Bates, 1960). Saturated salt solutions were changed every three days.

Twenty new second instar nymphs of *N. viridula* were placed in a round plastic container (64 x 118 mm) for each treatment and maintained in incubators for different climate conditions. The bugs were provided fresh green beans as food. The beans and containers were changed every two days. Data of nymphal mortality, number of moulting nymphs, adult mortality, mating date, oviposition date and date of nymph hatching were recorded at 10 am in the morning every day. Fresh body weight (g) at adult emergence of the bugs was taken using an electronic balance (Mettler Toledo, XP404S Precision Balance). In order to determine the frequency of adult mating, a permanent black marker pen was used to mark the scutellum or shoulder of male and female bugs while they were mating. Any mating bug (male or female) which performed a mating activity with a new individual (male or female) was considered to be part of a new mating pair. Egg-masses per female were calculated from the time of laying an egg-mass by dividing the total egg-masses by the total number of females from the time that they began to lay eggs. All egg-masses were counted under a microscope to determine the egg-mass size. The skin of egg-masses was taken off from the containers when the nymphs were at the 2nd instar stage and were examined under a microscope to determine egg-hatching ability.

Warming Tolerance (WT) and Thermal Safety Margins (TSM) were also tested as (Andrew *et al.* 2013; Deutsch *et al.* 2008, Diamond *et al.* 2012) they characterise the geographic covariances of insect thermal performance curves and climate (Angilletta 2009). Deutsch *et al.* (2008) defined warming tolerance (WT) is the difference between critical thermal maximum (CT_{max}) and the current climatological temperature of organism's habitat (T_{hab}). The critical thermal maxima (CT_{max}) of *N. viridula* populations from inland and coastal environments was 45.9°C (Chantry *et al.*, 2012). Habitat temperatures of *N. viridula* were collected from inland was 31.5°C (station 55008) and coastal location was 29.7°C (station 58077) during the summer period in 2010.

Statistical analysis

The data for developmental stages, and reproductive performance of *N. viridula* were examined by a 3-way Analysis of Variance (ANOVA) using IRRISTAT for Windows 5.0 developed by the International Rice Research Institute (IRRI). Means were compared by the method of least significant

difference at the level of 5% (5% LSD). Furthermore, three variables of *N. viridula* developmental stages, nymphal duration, nymphal survival and adult

longevity were tested for significance of linear, quadratic and cubic effects using orthogonal contrasts in IRRISTAT for Windows 5.0.

RESULTS

Influence of temperature and humidity regimes on development of *Nezara viridula*

The mean number of days required by nymphs of *N. viridula* to complete their development (from 2nd instar to adults) varied greatly from one temperature regime to another and the time taken for nymphal Survival of *N. viridula* - nymphal stages to adult, decreased with temperature increases: the highest survival rate was observed at 25°C and significantly higher compared to 30°C, 33°C and 36°C. ($P < 0.0001$; Figure 2). Although at 33°C, some *N. viridula* were mating, they died before they were able to produce egg-masses. At 36°C, *N. viridula* exhibited no mating activities. *N. viridula* successfully mated at 25 and 30°C only. The incubation period at 25°C (6.5

development increased with lower temperature with a significant quadratic effect at 1% level ($P < 0.01$) (Figure 1). For each nymphal instar, the mean number of days was significantly greater in lower temperature compared to higher temperature (Table 1).

± 0.2 days) was significantly longer than at 30°C (3.0 ± 0.9 days) and the incubation period (3.2 ± 0.7 days) at the low humidity regime (40% RH) was significantly longer than at high humidity (80% RH) (1.5 ± 0.5 days) (Table 1). The first instar duration of *N. viridula* reared at 25°C (4.3 ± 0.1 days) was significantly longer than when reared at 30°C (0.3 ± 0.3 days) (Table 1).

Figure 1. The effect of temperature on nymphal duration (ND), from 2nd instar to adults of *Nezara viridula* (L.). No significant differences were exhibited among locations, so data from both inland and coastal locations have been pooled.

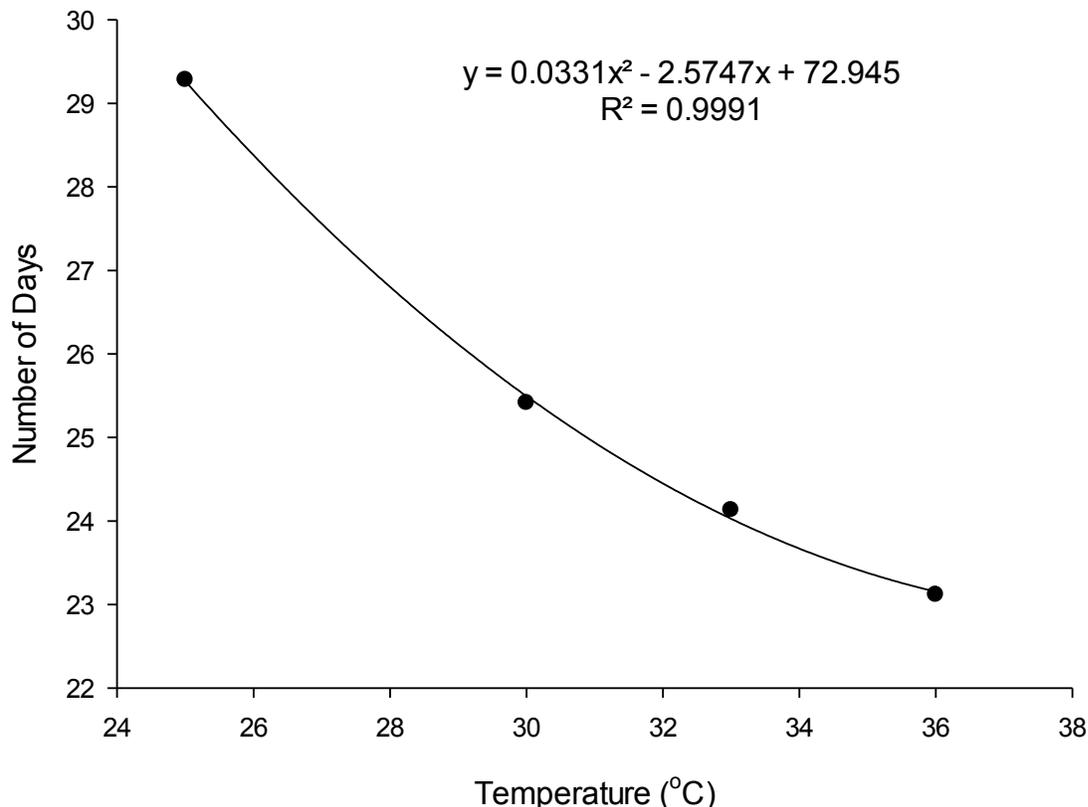
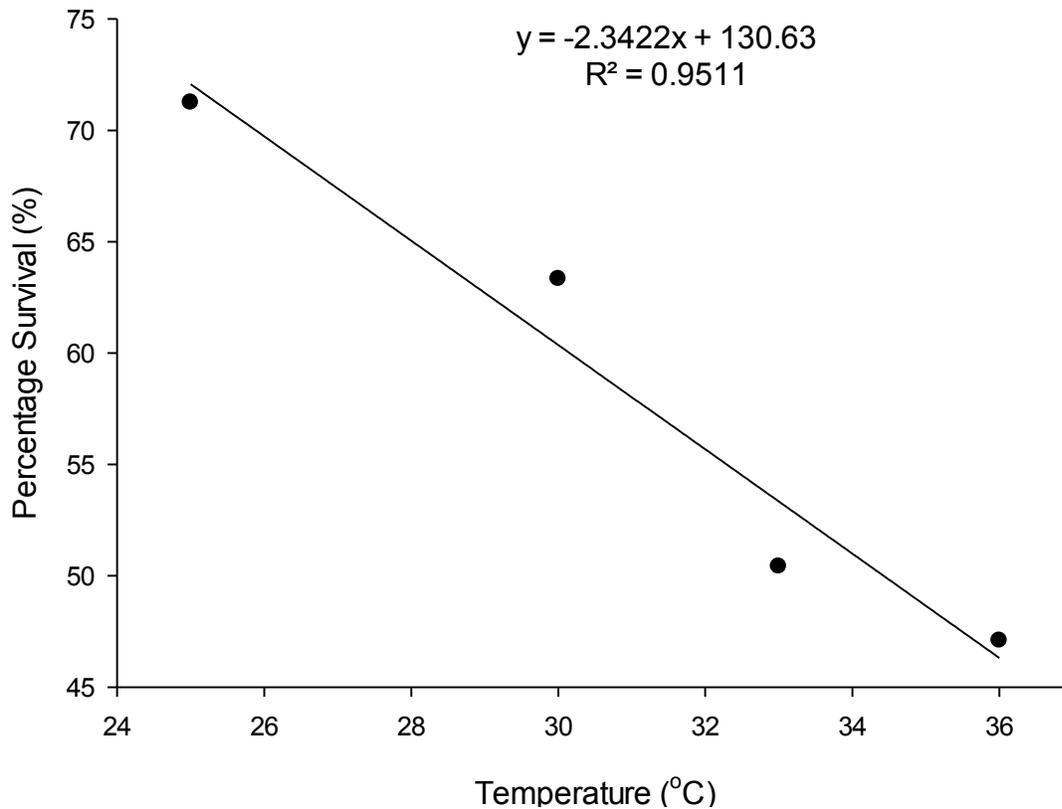


Figure 2. The effect of temperature on nymphal survival (NS), from 2nd instar to adults of *Nezara viridula* (L.). No significant differences were exhibited among locations, so data from both inland and coastal locations have been pooled.



The mean longevity of *N. viridula* adults varied widely between temperatures. The longevity of *N. viridula* adults was significantly shorter with increasing temperature with a quadratic effect significant at the 5% level ($P < 0.05$) (Figure 3). At the same temperature regime, the longevity of females was found to be longer than males (Table 1). At 25°C, *N. viridula* females could live up to 60.7 ± 6.7 days. This was longer than that of males (46.3 ± 5.5 days). At 30°C female longevity was 59.4 ± 7.8 days and male 41.6 ± 7.7 days while at 33°C female was 39.0 ± 5.6 days and male 30.2 ± 5.3 days; except at 36°C, the longevity of females was slightly shorter than males. Humidity regimes also had a significant impact on the longevity of *N. viridula*. The longevity (51.2 ± 5.2 days) of *N. viridula* reared at low humidity was significantly longer than when reared at high humidity (25.8 ± 3.1 days). At the same humidity regimes, females lived longer than males (Table 1).

Body weight of newly emerged adults was measured. At the same temperature or humidity regime, the body weight of *N. viridula* females was heavier than that of males (Table 2). The body weight of adults at 25°C ($0.14 \pm 0.00g$) and 30°C ($0.14 \pm 0.00g$) was significantly heavier compared to the body weight of *N. viridula* reared at 33°C ($0.12 \pm 0.00g$) and 36°C ($0.10 \pm 0.00g$), between which body weight at 33°C was significantly heavier than at 36°C. Humidity regimes had a significant impact on body weight of *N. viridula* adults. The significantly heavier body weight ($0.13 \pm 0.00g$) of *N. viridula* was observed at low humidity compared to body weight ($0.12 \pm 0.00g$) at high humidity (Table 2).

Table 1. The effect of temperature or humidity regimes on nymphal duration of each instar, male and female longevity and egg hatching duration of *Nezara viridula* (L.).

Climate condition	Mean duration \pm SE of nymphal stages (days)				Mean longevity \pm SE of adult <i>N. viridula</i> (days)		Incubation period (days)	1 st instar duration (days)
	2nd	3rd	4th	5th	Female	Male		
Temperature (°C)								
25	6.8 \pm 0.2 a	5.4 \pm 0.1 a	6.2 \pm 0.2 a	10.8 \pm 0.3 a	60.7 \pm 6.7 a	46.3 \pm 5.5 a	6.5 \pm 0.2 a	4.3 \pm 0.1 a
30	5.4 \pm 0.2 b	4.4 \pm 0.1 b	5.5 \pm 0.1 b	10.1 \pm 0.3 ab	59.4 \pm 7.8 a	41.6 \pm 7.7 a	3.0 \pm 0.9 b	0.3 \pm 0.3 b
33	5.1 \pm 0.2 b	3.8 \pm 0.1 c	5.1 \pm 0.2 c	10.2 \pm 0.4 ab	39.0 \pm 5.6 b	30.2 \pm 5.3 b	-	-
36	4.4 \pm 0.2 c	4.0 \pm 0.1 c	5.3 \pm 0.2 bc	9.5 \pm 0.2 b	10.6 \pm 2.3 c	14.8 \pm 2.2 c	-	-
Humidity (%)								
40	5.5 \pm 0.2 a	4.5 \pm 0.2 a	5.6 \pm 0.1 a	10.3 \pm 0.2 a	55.1 \pm 6.2 a	45.9 \pm 4.5 a	3.2 \pm 0.7 a	1.3 \pm 0.4 a
80	5.4 \pm 0.2 a	4.2 \pm 0.1 b	5.5 \pm 0.1 a	10.0 \pm 0.3 a	29.7 \pm 4.2 b	20.6 \pm 2.8 b	1.5 \pm 0.5 b	1.0 \pm 0.4 b

Mean \pm standard error (SE). Mean followed by different letters in the same column are significantly different at $p = 0.05$, using IRRISTAT program for Windows 5.0.

Means were compared by the method of least significant differences (LSD) at 5% level.

Table 2. The effect of temperature or humidity regimes on body weight, reproductive performance and egg hatchability of *Nezara viridula* (L.).

Climate condition	Mean weight (g) \pm SE of adult <i>N. viridula</i>			Mating frequency (times)	Pre-mating period (days)	Pre-oviposition period (days)	Egg-masses/female	Egg-mass size (eggs/egg mass)	Fecundity (Eggs/female)	% of egg mass hatched	Egg hatch-ability (%)
	Female	Male	Adults (F & M)								
Temperature (°C)											
25	0.16 \pm .00 a	0.12 \pm .00 a	0.14 \pm .00 a	6.2 \pm 0.6 a	25.8 \pm 1.8 a	50.1 \pm 2.1 a	1.8 \pm 0.3 a	62.6 \pm 2.8 a	113.9 \pm 15.4 a	82.6 \pm 6.0 a	52.7 \pm 10.9 a
30	0.15 \pm .01 a	0.12 \pm .00 a	0.14 \pm .00 a	2.1 \pm 0.6 b	33.4 \pm 6.7 a	39.2 \pm 12.0 b	0.5 \pm 0.2 b	17.7 \pm 5.4 b	17.8 \pm 9.0 b	41.2 \pm 13.5 b	4.8 \pm 1.8 b
33	0.13 \pm .00 b	0.10 \pm .01 b	0.12 \pm .00 b	0.3 \pm 0.1 c	11.5 \pm 6.6 b	-	-	-	-	-	-
36	0.11 \pm .00 c	0.09 \pm .00 b	0.10 \pm .00 c	-	-	-	-	-	-	-	-
Humidity (%)											
40	0.14 \pm .00 a	0.12 \pm .00 a	0.13 \pm .00 a	2.7 \pm 0.7 a	22.6 \pm 4.9 a	32.2 \pm 7.1 a	0.7 \pm 0.2 a	25.5 \pm 5.9 a	41.4 \pm 12.9 a	36.9 \pm 8.3 a	6.6 \pm 1.7 b
80	0.14 \pm .00 a	0.11 \pm .00 b	0.12 \pm .00 b	1.6 \pm 0.5 b	12.8 \pm 3.2 b	12.5 \pm 4.6 b	0.4 \pm 0.2 b	14.6 \pm 5.3 b	24.5 \pm 9.9 b	25.0 \pm 9.0 b	22.1 \pm 8.0 a

F – female, M – male, Mean \pm standard error (SE), Mean \pm standard error (SE). Mean followed by different letters in the same column are significantly different at $p = 0.05$, using IRRISTAT program for Windows 5.0.

Means were compared by the method of least significant differences (LSD) at 5% level.

Table 3. The interactions of temperature and humidity regimes on nymphal duration, survival, adult longevity and egg hatching duration of *Nezara viridula* (L.).

Tem. (°C)	Hum. (%)	Mean duration ± SE of nymphal stages (days)				Nymphal duration* (days)	Nymphal survival (%)	Mean longevity ± SE of adult <i>N. viridula</i> (days)			Incubation period (days)	1 st instar duration (days)
		2nd	3rd	4th	5th			Female	Male	Adults (F&M)		
25	40	6.8 ± 0.2 a	5.7 ± 0.1 a	6.5 ± 0.1 a	11.1 ± 0.3 a	30.2 ± 0.6 a	75.8 ± 0.8 a	76.1 ± 8.4 a	55.6 ± 8.4 ab	72.1 ± 7.5 a	7.0 ± 0.1 a	4.6 ± 0.1 a
25	80	6.8 ± 0.2 a	5.1 ± 0.2 a	5.9 ± 0.3 a	10.5 ± 0.5 abc	28.4 ± 1.1 a	66.7 ± 3.8 a	45.4 ± 5.5 a	37.0 ± 5.1 c	41.5 ± 4.9 bc	6.0 ± 0.1 b	3.9 ± 0.1 a
30	40	5.4 ± 0.1 a	4.4 ± 0.1 a	5.5 ± 0.1 a	10.5 ± 0.2 abc	25.7 ± 0.4 a	78.3 ± 5.1 a	77.8 ± 5.5 a	64.0 ± 6.8 a	68.1 ± 5.2 a	5.9 ± 0.4 b	0.5 ± 0.5 a
30	80	5.5 ± 0.4 a	4.4 ± 0.2 a	5.5 ± 0.2 a	9.7 ± 0.6 cd	25.1 ± 0.4 a	48.3 ± 4.2 a	40.9 ± 10.2 a	19.3 ± 3.6 d	31.9 ± 5.2 cd	-	-
33	40	5.1 ± 0.2 a	4.0 ± 0.1 a	5.0 ± 0.2 a	9.6 ± 0.5 cd	23.8 ± 0.6 a	61.7 ± 2.1 a	52.1 ± 7.7 a	44.4 ± 4.5 bc	47.2 ± 4.9 b	-	-
33	80	5.0 ± 0.2 a	3.5 ± 0.1 a	5.1 ± 0.3 a	10.8 ± 0.6 ab	24.5 ± 0.9 a	39.2 ± 7.9 a	25.9 ± 3.0 a	16.0 ± 4.5 d	21.3 ± 3.1 de	-	-
36	40	4.5 ± 0.3 a	4.0 ± 0.1 a	5.2 ± 0.3 a	10.0 ± 0.3 bcd	23.7 ± 0.4 a	52.5 ± 8.8 a	14.4 ± 4.0 a	19.7 ± 3.0 d	17.4 ± 3.0 e	-	-
36	80	4.2 ± 0.1 a	4.0 ± 0.2 a	5.3 ± 0.1 a	9.0 ± 0.3 d	22.5 ± 0.3 a	41.7 ± 6.5 a	6.7 ± 1.2 a	9.9 ± 1.9 d	8.6 ± 1.0 e	-	-

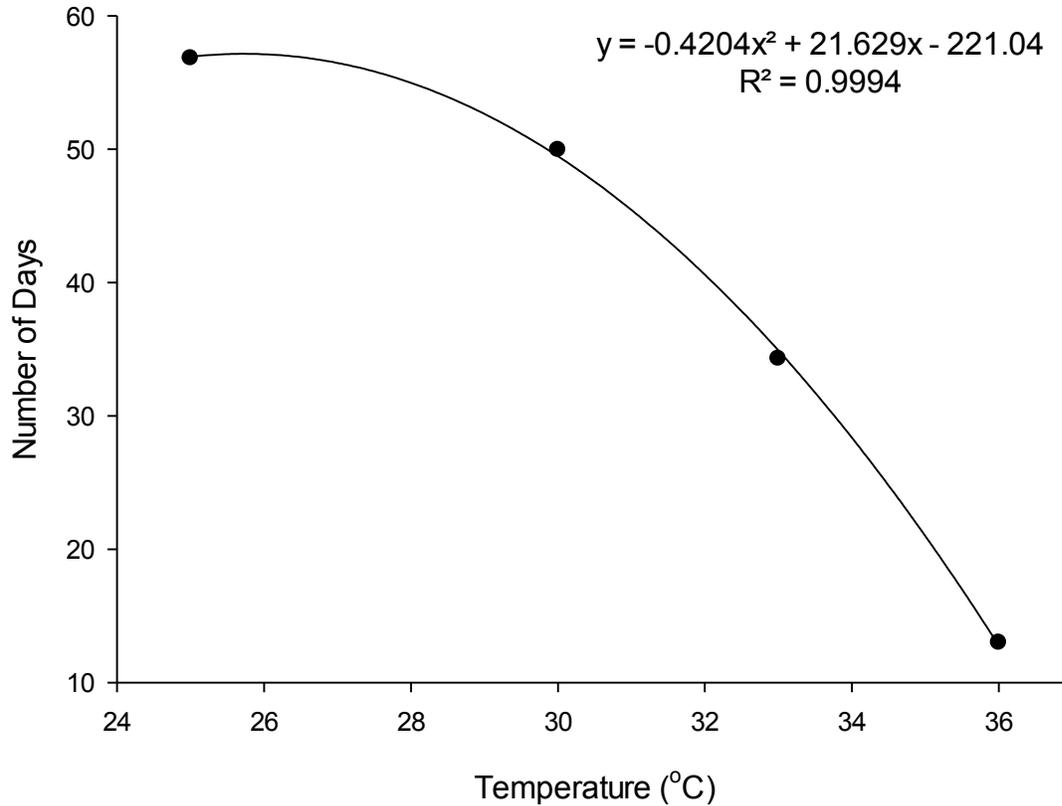
Tem – temperature, Hum – humidity. * – days from second instar of nymphs to adults. Mean ± standard error (SE). Mean followed by different letters in the same column are significantly different at p = 0.05, using IRRISTAT program for Windows 5.0. Means were compared by the method of least significant differences (LSD) at 5% level.

Table 4. The interaction effects of temperature and humidity regimes on body weight and reproductive performance and egg hatchability of *Nezara viridula* (L.).

Tem. (°C)	Hum. (%)	Mean weight (g) ± SE of adult <i>N. viridula</i>			Mating frequency (times)	Pre-mating period (days)	Pre-oviposition period (days)	Egg-masses/ female	Egg-mass size (eggs/egg mass)	Fecundity (Eggs/female)	% of egg mass hatched	Egg hatch-ability (%)
		Female	Male	Adults (F & M)								
25	40	0.16 ± .01 a	0.12 ± .00 a	0.14 ± .01 a	7.3 ± 0.7 a	28.6 ± 2.2 b	50.3 ± 1.9 b	2.0 ± 0.4 a	66.7 ± 5.2 a	129.8 ± 24.4 a	65.1 ± 6.0 c	17.0 ± 2.0 b
25	80	0.16 ± .00 a	0.12 ± .00 a	0.14 ± .00 a	5.0 ± 0.8 b	23.0 ± 2.5 bc	49.9 ± 3.9 b	1.7 ± 0.3 a	58.6 ± 1.1 b	98.0 ± 18.7 a	100.0 ± 0.0 a	88.4 ± 2.1 a
30	40	0.16 ± .00 a	0.13 ± .00 a	0.14 ± .00 a	3.2 ± 1.0 c	51.1 ± 4.2 a	78.5 ± 4.4 a	1.0 ± 0.4 a	35.4 ± 1.9 c	35.7 ± 15.0 a	82.4 ± 11.3 b	9.6 ± 2.4 c
30	80	0.14 ± .01 a	0.12 ± .00 a	0.13 ± .01 a	1.0 ± 0.5 d	15.8 ± 7.2 bc	-	-	-	-	-	-
33	40	0.13 ± .00 a	0.12 ± .00 a	0.13 ± .00 a	0.2 ± 0.2 d	10.7 ± 10.7 c	-	-	-	-	-	-
33	80	0.13 ± .01 a	0.09 ± .01 a	0.11 ± .00 a	0.3 ± 0.2 d	12.3 ± 8.7 c	-	-	-	-	-	-
36	40	0.11 ± .00 a	0.09 ± .01 a	0.10 ± .00 a	-	-	-	-	-	-	-	-
36	80	0.11 ± .01 a	0.10 ± .00 a	0.10 ± .00 a	-	-	-	-	-	-	-	-

Tem – temperature, Hum – humidity, F – female, M – male, Mean ± standard error (SE). Mean followed by different letters in the same column are significantly different at p = 0.05, using IRRISTAT program for Windows 5.0. Means were compared by the method of least significant differences (LSD) at 5%

Figure 3. The effect of temperature on adult longevity (ADL) of *Nezara viridula* (L.). No significant differences were exhibited among locations, so data from both inland and coastal locations have been pooled.



The mating frequency of *N. viridula* varied greatly from one temperature to other. At 25°C, mating frequency of *N. viridula* (6.2 ± 0.6 times) was significantly higher than at 30°C (2.1 ± 0.6 times) and 33°C (0.3 ± 0.1 times), between which mating frequency at 30°C was highly significantly different (Table 2). Mating activity of *N. viridula* was not observed at 36°C, possibly due to suppression by higher temperature or the short adult longevity of *N. viridula* (9.7 ± 0.1 to 17.6 ± 3.5 days for Breeza and 7.5 ± 2.0 to 17.1 ± 5.8 days for Grafton) (Appendix 5). The mating frequency (2.7 ± 0.7 times) at the low humidity regime was significantly higher than at the high humidity regime (1.6 ± 0.5 times) (Table 2).

There was a significant effect of temperature on the duration of the pre-mating period of *N. viridula*. The pre-mating period of *N. viridula* (11.5 ± 6.6 days) at

33°C was significantly shorter compared to the pre-mating period at 25°C (25.8 ± 1.8 days) and 30°C (33.4 ± 6.7 days), between which pre-mating periods were not significantly different. The pre-mating period of *N. viridula* reared in low humidity (22.6 ± 4.9 days) was significantly longer than at the high humidity regime (12.8 ± 3.2 days) (Table 2).

Pre-oviposition period, number of egg-masses per female, size of egg-mass (number of eggs per egg-mass), fecundity (eggs per female), percentage of egg-mass hatched, and egg hatchability of *N. viridula* were found to be significantly longer or greater at 25°C and/or low humidity than at 30°C and/or high humidity, except for the egg hatchability at the low humidity regime, $6.6 \pm 1.7\%$ which was significantly lower than at high humidity, $22.1 \pm 8.0\%$ (Table 2).

Interactions of temperature and humidity on development of *Nezara viridula*

The interactions between temperature and humidity had a significant effect on incubation period. Incubation period (7.0 ± 0.1 days) at 25°C with low humidity was significantly longer compared to incubation period at 25°C with high humidity (6.0 ± 0.1 days) and at 30°C with low humidity (5.9 ± 0.4 days), between which incubation period was not significantly different (Table 3).

The interactions between temperature and humidity had a significant impact on male longevity. The longevity of adults (females and males pooled together) at 25°C (72.1 ± 7.5 days) and 30°C (68.1 ± 5.2 days) with low humidity regime was significantly longer than at 25°C (41.5 ± 4.9 days) and 30°C (31.9 ± 5.2 days) with high humidity. Significantly shorter longevity of adults was recorded at 36°C, 17.4 ± 3.0 days and 8.6 ± 1.0 days with low and high humidity regimes, respectively. It is important to note that, at the same temperature, the longevity of *N. viridula* adults with low humidity was longer than with the high humidity (Table 3).

The interactions between temperature and humidity significantly changed reproductive performance of *N. viridula* (Table 4). Greater mating frequency of *N. viridula* was recorded at 25°C with low and high humidity regimes. Mating frequency (7.3 ± 0.7 times) at 25°C with 40% RH was significantly higher than at 25°C with 80% RH (5.0 ± 0.8 times) (Table 4). The mating frequency of *N. viridula* at 25°C with low and high humidity was significantly higher compared to mating frequency at 30°C and 33°C with low and high humidity. Pre-mating period of *N. viridula* at 30°C with 40% RH (51.1 ± 4.2 days) was significantly longer compared to pre-mating period at 25°C with 40% RH (28.6 ± 2.2 days). The shortest pre-mating period of *N. viridula* was recorded at 33°C with low humidity (10.7 ± 10.7 days) and high humidity (12.3 ± 8.7 days) (Table 4).

Pre-oviposition period was longer for females reared at 30°C with 40 % RH (78.5 ± 4.4 days) than those reared at 25°C with 40% RH (50.3 ± 1.9 days) and with 80% RH (49.9 ± 3.9 days), between which pre-oviposition period was not significantly affected. Larger egg-mass size was recorded at low temperature. At 25°C with 40% RH, egg-mass size of *N. viridula* (66.7 ± 5.2 eggs/egg-mass) was significantly larger than egg-mass size of *N. viridula* reared at 25°C with 80% RH (58.6 ± 1.1 eggs/egg-mass) and at 30°C with 40% RH (35.4 ± 1.9 eggs/egg-mass). Egg-mass size at 25°C with 80% RH

was significantly greater than at 30°C with 40% RH (Table 4).

Egg-masses of *N. viridula* reared at 25°C with 80% RH hatched at optimum levels ($100 \pm 0.0\%$) compared to egg-masses kept at 25°C with 40% RH ($65.1 \pm 6.0\%$) and at 30°C with 40% RH ($82.4 \pm 11.3\%$) (Table 4). Egg hatchability of *N. viridula* was significantly higher when kept at 25°C with 80% RH ($88.4 \pm 2.1\%$) compared to egg hatchability at 25°C with 40% RH ($17.0 \pm 2.0\%$) and at 30°C with 40% RH ($9.6 \pm 2.4\%$), between which egg hatchability at 25°C with 40% RH was higher than at 30°C with 40% RH (Table 4). The interactions of temperature and humidity had no effect on egg-mass per female and fecundity of *N. viridula* females (Table 4).

Interactions of location and temperature on development of *Nezara viridula*

Incubation period, pre-mating period, pre-oviposition period, egg-mass size and fecundity of *N. viridula* all exhibited a significant interaction between location and temperature (Appendix 1 and 2). For the same temperature regimes, at 30°C the incubation period of inland *N. viridula* (3.4 ± 1.5 days) was significantly longer compared to incubation period of coastal *N. viridula* (2.5 ± 1.1 days) at 30°C (Appendix 1).

The pre-mating period of inland *N. viridula* at 30°C (24.3 ± 11.3 days) was significantly shorter compared to coastal *N. viridula* (42.5 ± 5.9 days) (Appendix 2). Pre-oviposition period of *N. viridula* was different between locations. The mean number of days required by inland *N. viridula* from newly emerged adults to the day of oviposition at 25°C (52.6 ± 1.3 days) was significantly longer compared to coastal *N. viridula* at the same temperature (47.6 ± 3.8 days), while pre-oviposition period of inland *N. viridula* at 30°C (34.6 ± 15.5 days) was shorter than those from coastal *N. viridula* (43.9 ± 19.7 days) (Appendix 2). The mean number of egg-masses per female of inland *N. viridula* at 25°C (1.2 ± 0.2 egg-mass/female) was significantly lower than those from coastal *N. viridula* (2.4 ± 0.3 egg-mass/female). The fecundity of coastal *N. viridula* females at 25°C (146.0 ± 19.8 eggs/female) was significantly greater compared to fecundity of inland *N. viridula* (81.8 ± 15.6 eggs/female) (Appendix 2).

Interactions of location and humidity on development of *Nezara viridula*

The interactions of location and humidity were significant for incubation period, and egg-mass size of *N. viridula* (Appendix 3 and 4). The mean number of days required from oviposition to hatching

incubation period) by inland *N. viridula* (3.4 ± 1.0 days) at 40% RH was significantly longer compared to incubation period of coastal *N. viridula* (3.0 ± 0.9 days) at 40% RH (Appendix 3). The mean number of eggs per egg-mass (egg-mass size) of inland *N. viridula* at 40% RH (27.9 ± 9.4 eggs/egg-mass) was significantly higher compared to egg-mass size of coastal *N. viridula* (23.1 ± 7.6 eggs/egg-mass) at 40% RH (Appendix 4).

Interactions of location, temperature and humidity on development of *Nezara viridula*

Four developmental stages of *N. viridula*, 2nd instar duration, incubation period, male longevity and pre-oviposition period, exhibited a significant interactions between temperature and humidity regimes between two locations (Appendix 5 and 6). The 2nd instar duration of inland and coastal *N. viridula* at the same temperature and humidity regimes was significantly different, with the exception of those reared at 30°C with 80% RH. The 2nd instar duration of inland *N. viridula* (5.0 ± 0.1 days) at 30°C with 80% RH was significantly shorter compared to coastal *N. viridula* (6.0 ± 0.7 days) at 30°C with 80% RH, (Appendix 5). Incubation period of inland *N. viridula* reared at 30°C with 40% RH (6.8 ± 0.2 days) was significantly longer compared to coastal *N. viridula* at 30°C with 40% RH (5.0 ± 0.0 days) (Appendix 5).

Pre-oviposition period of inland and coastal *N. viridula* at 30°C with 40% RH (inland, 69.1 ± 1.7 days and coastal, 87.8 ± 2.4 days) was significantly longer compared to pre-oviposition period of inland and coastal *N. viridula* at 25°C with 40% or 80% RH (inland, 53.6 ± 2.1 days (40% RH), 51.6 ± 1.7 days (70% RH) and coastal, 47.0 ± 1.6 days (40% RH), 48.2 ± 8.3 days (70% RH). Pre-oviposition period of inland *N. viridula* at 30°C with 40% RH (69.1 ± 1.7 days) was significantly shorter compared to coastal *N. viridula* (87.8 ± 2.4 days) at 30°C with 40% RH, (Appendix 6).

Warming Tolerance (WT) and Thermal Safety margin (TSM)

The WT of inland *N. viridula* was 14.4°C (WT = $CT_{max} - T_{hab} = 45.9^{\circ}C - 31.5^{\circ}C$) and WT of coastal *N. viridula* was 16.2°C (WT = $45.9^{\circ}C - 29.7^{\circ}C$). The TSM of inland *N. viridula* was -6.3°C (TSM = $25^{\circ}C - 31.5^{\circ}C$) and TSM of coastal *N. viridula* was -4.7°C (TSM = $25^{\circ}C - 29.7^{\circ}C$).

DISCUSSION

Influence of temperature and humidity on development of *Nezara viridula*

Here, we assessed the impacts of predicted climate change scenarios on development of *N. viridula*. Insect development is highly influenced by both temperature and humidity. This may be from direct impacts of both on terrestrial insects within a single generation or from long-term exposure at different climatic regions from where the organisms have bred and successfully continued multiple generations. The effects of temperature on insect development may vary among species, with lower temperatures typically resulting in a decrease in rate of development, and a lengthening of the period of insect development; but high temperature shortens the duration of time spent in each developmental stage (Hintze, 1970; Ross *et al.*, 1982). Results from this study indicated that the developmental time of *N. viridula* nymphal stages (from 2nd instar to adults) and adult longevity declined with increasing temperature. However, the rate of nymphal survival decreased with increasing temperature or humidity regimes (Table 1). The current results showed that the nymphal duration (from 2nd instar to adults) at 25°C (29.3 ± 0.7 days) was longer than at 30°C (25.4 ± 0.3 days), 33°C (24.1 ± 0.5 days), and 36°C (23.1 ± 0.3 days). These results are similar to previous studies which reported that the mean nymphal development period of *N. viridula* when reared at 25-27°C, 55-65% RH, and 14 h. photophase was 31.8 days, nymphal duration of 1st instar was 3.8 days, 2nd instar (5.2 days), 3rd instar (4.5 days), 4th instar (6.4 days) and 5th instar (11.9 days) (or from 2nd instar to adults took 28.0 days) (Harris and Todd 1980). Harris and Todd's (1980) findings show *N. viridula* nymphal development period was 1.3 days shorter than the results in this study. This difference may be explained in terms of different host plants or food crops. In this study, fresh green bean pods (*P. vulgaris*) were provided to *N. viridula* as food, whereas Harris and Todd (1980) provided fresh green beans (*P. vulgaris*) and green shelled peanuts (*Arachis hypogaea*) to *N. viridula*. There have been many reports of rearing *N. viridula* with seeds. Panizzi and Saraiva (1993) reported nymphal duration was 26.0 days at 25°C on immature soybean pods and 39.3 days on immature radish fruits. In addition, Jones Jr. and Brewer (1987) reported that the same nymphal duration was 22.7 days at 27°C on green beans and peanuts. There is evidence that diets derived from different plant species could have a great effect on rates of development, survival and reproduction of *N. viridula* (Panizzi 1997, Velasco *et al.* 1995). The optimum temperature for *N. viridula* development appears to be 25°C, since all stages of *N. viridula* at this temperature developed successfully with a higher rate of survival, higher reproduction

(large egg-mass size and high fecundity) and higher percentage of egg hatchability compared to 30°C. Moreover, at 33 and 36°C, the rate of nymphal survival and mating frequency were very low and *N. viridula* were unable to develop well, causing short adult longevity and inability to lay eggs. The results obtained here were similar to those of Velasco and Walter (1993) who studied the influence of fluctuating temperature on egg production by *N. viridula* and on survival and development of nymphs. Nymphal survival to adult stage was low (32.9%) under the high temperature regime (27/37°C) and none of the resulting adults survived long enough to mate and reproduce.

Adult longevity and weight declined with increasing temperature and/or humidity regimes. Generally, females lived longer and were heavier than males at the same temperature or humidity regimes (Table 1). Previous studies have suggested an optimum temperature for *N. viridula* of about 25°C (Ali and Ewiess 1977). Thus high temperatures (30, 33, and 36°C) had adverse effects on the development and reproductive capacity of *N. viridula*. At 25°C, *N. viridula* had more frequent mating, 6.2 ± 0.6 times during their life span, many egg-masses per female, larger egg-mass, high fecundity and high egg hatchability compared to 30°C. Moreover, at temperatures higher than 30°C (e.g., 33 and 36°C), *N. viridula* was unable to reproduce or failed to lay any eggs (Table 2).

High temperature might cause temporary or permanent sterility, or deactivation of sperm stored in the spermatheca resulting in reduced fertility (Riordan, (1957)) and may explain the results in this study. Females of *N. viridula* failed to lay eggs at 30°C and higher. Ju *et al.* (2011) demonstrated that females of the sycamore lace bug, *Corythucha ciliaca* (Hemiptera: Tingidae) failed to lay eggs at 16°C, suggesting that low temperatures also induced sterility. Ju *et al.* (2011) suggested that both lower and higher temperatures would lead to developmental stagnancy of the ovaries.

Our results indicated that mating frequency, pre-mating period, pre-oviposition, egg-mass per female, egg-mass size, fecundity and percentage of egg-mass hatched responded positively to decreasing humidity regimes (Table 2). However the high humidity regime improved egg hatchability of *N. viridula*. Small percentages of egg-masses and a low percentage of eggs per egg-mass hatched in low humidity compared to high humidity because desiccation may affect the egg development and result in low emergence rate or

egg hatchability of *N. viridula*. Harris and Todd (1980) cited several studies where egg and larval development were accelerated at high humidity. Most terrestrial insect embryos support metabolism with oxygen from the environment by diffusion across the eggshell. Because metabolism is more temperature sensitive than diffusion, embryos should be relatively oxygen-limited at high temperature or temperature strongly affects egg metabolic rates (Woods and Hill 2004). In different life stages of terrestrial insects, eggs are usually sensitive to oxygen-water tradeoffs. Eggs are particularly immobilized in their oviposition microsite, whereas juveniles and adults may search out water and mobile life stages also possess rapid-acting systems for regulating oxygen flux and water balance (Woods and Harrison 2001). Insect eggs should be sensitive to oxygen-water tradeoff, because they are unable to forage for water, having high surface area-to-volume ratio, and experience large temperature driven changes in oxygen demand (Zrubek and Woods 2006).

Interactions of temperature and humidity on development of *Nezara viridula*

Generally, the mean nymphal duration, survival and adult longevity with low humidity was longer or higher compared to high humidity at the same temperature. Moreover, the reproductive performance of *N. viridula* such as mating frequency, pre-mating period and egg-mass size with low humidity were longer or larger than at high humidity at the same temperature. These results indicated that *N. viridula* were unable to develop well at a high humidity. However, high humidity improved the percentage of egg-mass hatched and egg hatchability of *N. viridula*. At 30°C with high humidity and 33°C (or over 30°C) with low and high humidity, no insects survived long enough to reproduce or to lay their eggs. These results indicated that the above climate conditions were unfavourable for the development and reproduction of *N. viridula*.

Interactions of location and temperature on development of *Nezara viridula*

The interactions of location and temperature had no effect on nymphal duration, nymphal survival, adult longevity or body weight of *N. viridula*. These results showed that the life cycle of inland and coastal populations of *N. viridula* are similar at the same temperature regimes. The average annual maximum temperature is similar at both sites (26°C) however minimum temperature is higher at the coastal site (Grafton, 13.7°C) compared to the inland site (Breeza, 10.9°C) (BoM 2011).

Interactions of location and humidity had no effect on the development and reproduction of *N. viridula* between the inland and coastal populations. These results can potentially be explained by the observation that, although coastal Grafton is more humid with a mean 3 pm relative humidity of 53% compared to inland Breeza at 46%, this variation is less than 10% and is probably not to be a big enough difference to have an effect.

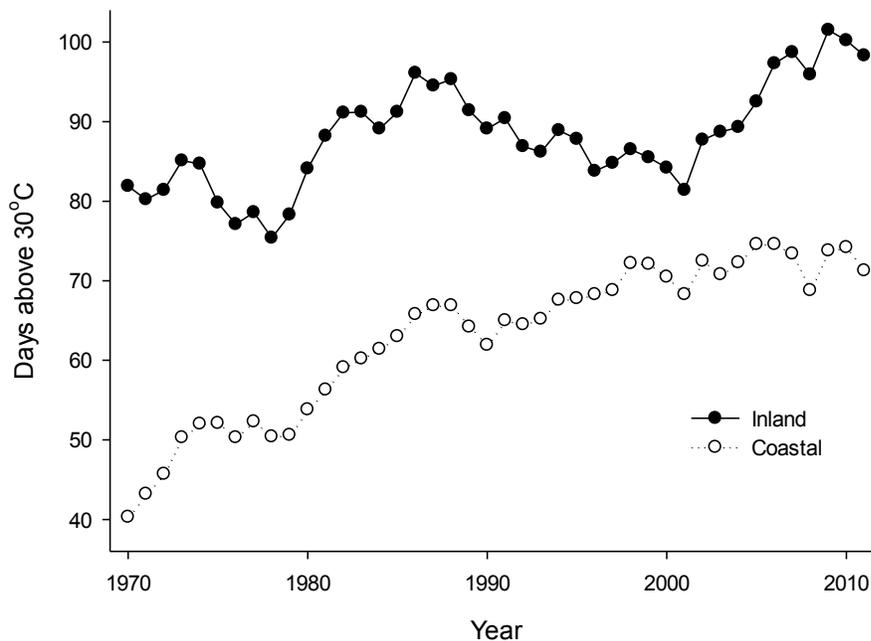
Interactions of location, temperature and humidity on development of *Nezara viridula*

The nymphal duration, survival, adult longevity, body weight (insect fitness) and reproductive performance of *N. viridula* were not significantly different for interactions of location, temperature and humidity regimes. Average annual maximum temperature is similar at both sites (26°C) but the minimum temperature is higher at coastal (13.7°C) compared to inland (10.9°C) (BoM 2011).

With climate change, global average surface temperature is expected to increase by 1.4-5.8°C by 2100 with atmospheric carbon dioxide (CO₂) concentrations expected to rise to between 540 and 970 ppm over the same period (Houghton *et al.* 2001, IPCC 2007). In the inland North West region

(Breeza) of NSW, the climate is highly likely to be hotter in all seasons by 2050, with the greatest warming in spring and winter. Average daily maximum and minimum temperatures are very likely to increase by between 1 and 3°C in different parts of the region. On the other hand, in the north coast region) of NSW, the average maximum temperature is expected to increase 1.0-1.5°C in summer and 2.0-3.0°C in winter and average daily minimum temperature are projected to increase 2.0-3.0°C by 2050 in all seasons (NSW Climate Impact Profile 2010). Recent inland and coastal mean summer maximum temperatures are 31.5°C and 29.7 respectively (BoM 2011). In this context, if the temperature increased further under global climate change, coastal and inland mean summer maximum temperature will be over 30°C by 2050. More extreme events such as extreme high temperatures are also predicted (Easterling *et al.* 2000), which will increase the impact of higher temperature. The number of days above 30°C at the inland site increased from 82 in 1970 to 98 in 2011 and based on this trend there could be 109 days above 30°C in 2050. At the coastal site, the number of days above 30°C increased from 40 in 1970 to 71 in 2011 and based on this trend could be 105 days above 30°C in 2050 (Figure 4).

Figure 4. Ten-year moving averages for number of days per year exceeding 30°C based on Historical temperature (1970-2011) at inland (Breeza) and coastal (Grafton) sites.



With regard to results of these experiments, at a temperature of 30°C or higher, *N. viridula* had reduced reproductive performance (mating activities), fecundity; prolonged or delayed pre-mating period, and shortened life span of adults. However, at a temperature of 25°C across all humidity regimes, coastal and inland populations of *N. viridula* developed successfully with a high rate of nymphal survival, more frequent mating, higher fecundity (eggs/female) and higher egg hatchability (Appendix 6). This temperature is similar to current coastal and inland mean autumn maximum temperature (March to May) 25.8°C (coastal) and 26.1°C (inland) (BoM 2011). If the average autumn maximum temperature increased by 3°C by 2050, it would still be below 30°C at both coastal and inland sites. However, the increased frequency of extreme autumn temperature events >30°C could impact on development and reproduction of *N. viridula*.

Previous studies have suggested that optimum temperature for *N. viridula* is about 25°C (Ali and Ewiess 1977). It is important to note that in this study, populations of *N. viridula* were found to be high during the soybean maturity stage (the April to May autumn period) with temperature about 26°C (observation during collection of *N. viridula* for culture and experiment). This indicated that the autumn period is a favourable environmental condition for the development and reproduction of *N. viridula* compared to the summer (December to February) with mean summer maximum temperatures of 29.7°C (coastal site) and 31.5°C (inland site) (BoM 2011). These summer temperatures are more likely to be unfavourable for the development and reproduction of *N. viridula*. Thus, if climate change were to affect the region, including elevated temperatures more extreme events and a shift of the rainfall regime toward summer in inland and coastal sites, *N. viridula* may be unable to adapt immediately. *N. viridula* might require time to adapt to less favourable conditions or shift distribution depending on availability and suitability of food plants. On the other hand, it is possible that soybean growers may plant soybeans later in the future to avoid extreme summer temperatures and this would favour *N. viridula* by maintaining maximum temperatures at the optimum level during the soybean podding stage. The warming tolerance (WT) of inland populations of *N. viridula* was 14.4°C; compared to coastal populations of *N. viridula* at 16.2°C. This indicates both populations could tolerate temperature increases before their performance is reduced to negligible or lethal levels. However, thermal safety margin (TSM) of inland *N. viridula* was -6.5°C and coastal *N.*

viridula was -4.7°C. Both populations in summer are vulnerable already to the thermal limits of their habitat in summer exposing them to heat stress. To overcome this, *N. viridula* will need to use behavioural mechanisms to reduce exposure to heat stress, such as foraging and flying in the cooler parts of the day.

In Japan, *N. viridula* has long been known to occur in southern parts, but recently adults have been observed in Osaka, central Japan, at least 70 km further north than the northern limit of distribution reported in the early 1960s (Kiritani 1971, Kiritani *et al.* 1963, Musolin and Numata 2003). It is evident that temperatures in the present lab 20/30°C treatment would be equivalent to field conditions in south-eastern Queensland during summer: these temperatures affected duration of nymphal development of *N. viridula*. Moreover, fluctuating temperatures between of 27/37°C have adversely affected nymphal and adult performance and survivorship of *N. viridula*: no insects survived long enough to mate and reproduce (Velasco and Walter 1993). However, Velasco and Walter (Velasco and Walter 1993) concluded that low *N. viridula* nymphal densities in south-eastern Queensland during summer cannot be explained by ambient temperature conditions alone. Temperature, however, may have an indirect influence on the abundance of *N. viridula* through its influence on the availability and suitability of host plants. It is also important to note that the bugs, *N. viridula* were maintained in containers inside incubators with consistent temperature regimes throughout the assessment period (i.e. no diurnal fluctuation). On the other hand, the bugs were not able to use behavioural adaptations to modify the effects of the warming climate within their containers. For instance, the bugs did not have the ability to move to a cooler microclimate within the containers.

In conclusion, the speed and duration of insect development are dependent upon a combination of external and internal factors, of which temperature is a more important factor compared to humidity. High temperatures shorten, and low temperatures lengthen, the period of insect development and metamorphosis (Hintze 1970). The optimum temperature of *N. viridula* in development was 25°C and intermediate humidity about 40 ± 10% RH, since all developmental stages of *N. viridula* developed successfully with high rates of nymphal survival, adults living long enough to mate and reproduce large egg-masses with higher fecundity. These results show that shorter nymphal duration, shorter adult longevity,

of mating frequency and reproduction occurred in higher temperature conditions. The present results suggested that *N. viridula* could not adapt to climate extremes even for a short time period. *N. viridula* in this study required a certain period under unfavourable climate conditions for successful development and reproduction. For instance, from an evolutionary point of view, the cold adaptation seen in temperate populations may be the consequence of long-term directional selection such as in *Drosophila* (Ayrinhac *et al.* 2004). However, no differences in nymphal duration, survival, adult longevity and reproduction performance between inland and coastal *N. viridula* were found under different experimental climate conditions.

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Appendix 1. The interaction of locations and temperature regimes on nymphal duration, survival, adult longevity and egg hatching duration of *Nezara viridula* (L.)

Loc	Tem (°C)	Mean duration ± SE of nymphal stages (days)				Nymphal duration* (days)	Nymphal survival (%)	Mean longevity ± SE of adult <i>N. viridula</i> (days)			Incubation period (days)	1 st instar duration (days)
		2nd	3rd	4th	5th			Female	Male	Adults (F&M)		
I	25	7.1 ± 0.2 a	5.3 ± 0.2 a	6.5 ± 0.2 a	11.3 ± 0.4 a	30.2 ± 0.8 a	69.2 ± 6.1 a	68.0 ± 11.5 a	57.5 ± 8.2 a	64.8 ± 10.2 a	6.5 ± 0.2 a	4.4 ± 0.2 a
I	30	5.3 ± 0.2 a	4.3 ± 0.2 a	5.8 ± 0.1 a	10.7 ± 0.3 a	26.0 ± 0.3 a	64.2 ± 7.2 a	58.3 ± 11.4 a	40.6 ± 12.4 bc	50.6 ± 9.7 a	3.4 ± 1.5 b	-
I	33	5.1 ± 0.3 a	3.6 ± 0.2 a	5.3 ± 0.2 a	10.7 ± 0.7 a	24.6 ± 0.9 a	50.8 ± 6.2 a	40.6 ± 9.7 a	31.0 ± 4.5 c	35.6 ± 6.4 a	-	-
I	36	4.5 ± 0.4 a	3.9 ± 0.1 a	5.4 ± 0.3 a	9.4 ± 0.2 a	23.2 ± 0.5 a	48.3 ± 7.0 a	10.6 ± 3.2 a	16.3 ± 2.1 de	13.6 ± 2.4 a	-	-
C	25	6.6 ± 0.2 a	5.5 ± 0.2 a	6.0 ± 0.3 a	10.3 ± 0.4 a	28.4 ± 1.0 a	73.3 ± 6.9 a	53.5 ± 6.4 a	35.1 ± 3.7 bc	48.9 ± 6.7 a	6.5 ± 0.3 a	4.1 ± 0.2 a
C	30	5.6 ± 0.3 a	4.4 ± 0.1 a	5.3 ± 0.1 a	9.5 ± 0.4 a	24.8 ± 0.3 a	62.5 ± 9.0 a	60.4 ± 11.8 a	42.7 ± 10.2 b	49.4 ± 9.5 a	2.5 ± 1.1 c	0.5 ± 0.5 a
C	33	5.1 ± 0.2 a	4.0 ± 0.1 a	4.8 ± 0.2 a	9.8 ± 0.4 a	23.7 ± 0.6 a	50.0 ± 8.9 a	37.4 ± 6.4 a	29.4 ± 10.0 cd	32.9 ± 7.7 a	-	-
C	36	4.2 ± 0.1 a	4.0 ± 0.2 a	5.2 ± 0.1 a	9.6 ± 0.5 a	23.1 ± 0.4 a	45.8 ± 9.1 a	10.5 ± 3.7 a	13.4 ± 4.1 e	12.3 ± 3.5 a	-	-

Loc – locations, I – inland, C – coastal, Tem – temperature, * – days from second instar of nymphs to adults, Mean ± standard error (SE). Mean followed by different letters in the same column are significantly different at $p = 0.05$, using IRRISTAT program for Windows 5.0. Means were compared by the method of least significant differences (LSD) at 5% level.

Appendix 2. The interaction effects of locations and temperature regimes on body weight and reproductive performance and egg hatchability of *Nezara viridula* (L.)

Loc	Tem (°C)	Mean weight (g) ± SE of adult <i>N. viridula</i>			Mating frequency (times)	Pre-mating period (days)	Pre-oviposition period (days)	Egg-masses/female	Egg-mass size (eggs/egg mass)	Fecundity (Eggs/female)	% of egg mass hatched	Egg hatch-ability (%)
		Female	Male	Adults (F & M)								
I	25	0.16 ± .01 a	0.13 ± .00 a	0.14 ± .00 a	5.5 ± 0.7 a	25.9 ± 2.9 b	52.6 ± 1.3 a	1.2 ± 0.2 b	65.3 ± 5.6 a	81.8 ± 15.6 b	86.5 ± 6.4 a	54.4 ± 15.7 a
I	30	0.15 ± .01 a	0.12 ± .00 a	0.14 ± .01 a	2.0 ± 1.3 a	24.3 ± 11.3 b	34.6 ± 15.5 c	0.3 ± 0.2 c	19.2 ± 8.6 a	12.6 ± 8.0 c	40.0 ± 20.0 a	4.4 ± 2.9 a
I	33	0.13 ± .01 a	0.11 ± .01 a	0.12 ± .01 a	0.3 ± 0.2 a	19.3 ± 12.3 bc	-	-	-	-	-	-
I	36	0.11 ± .00 a	0.10 ± .00 a	0.11 ± .00 a	-	-	-	-	-	-	-	-
C	25	0.15 ± .01 a	0.12 ± .00 a	0.14 ± .00 a	6.8 ± 1.0 a	25.7 ± 2.4 b	47.6 ± 3.8 b	2.4 ± 0.3 a	60.0 ± 0.8 a	146.0 ± 19.8 a	78.6 ± 10.5 a	51.0 ± 16.4 a
C	30	0.15 ± .01 a	0.12 ± .00 a	0.13 ± .01 a	2.2 ± 0.4 a	42.5 ± 5.9 a	43.9 ± 19.7 b	0.7 ± 0.4 c	16.2 ± 7.3 a	23.1 ± 16.7 c	42.4 ± 20.1 a	5.2 ± 2.5 a
C	33	0.14 ± .00 a	0.10 ± .01 a	0.12 ± .00 a	0.2 ± 0.2 a	3.7 ± 3.7 c	-	-	-	-	-	-
C	36	0.11 ± .01 a	0.09 ± .01 a	0.10 ± .00 a	-	-	-	-	-	-	-	-

Loc – locations, I – inland, C – coastal, Tem – temperature, Mean ± standard error (SE). Mean followed by different letters in the same column are significantly different at p = 0.05, using IRRISTAT program for Windows 5.0. Means were compared by the method of least significant differences (LSD) at 5% level.

Appendix 3. The interaction of locations and humidity regimes on nymphal duration, survival, adult longevity and egg hatching duration of *Nezara viridula* (L.)

Loc	Hum. (%)	Mean duration ± SE of nymphal stages (days)				Nymphal duration* (days)	Nymphal survival (%)	Mean longevity ± SE of adult <i>N. viridula</i> (days)			Incubation period (days)	1 st instar duration (days)
		2nd	3rd	4th	5th			Female	Male	Adults (F&M)		
I	40	5.5 ± 0.3 a	4.4 ± 0.2 a	5.7 ± 0.2 a	10.4 ± 0.3 a	26.0 ± 0.9 a	63.3 ± 5.6 a	56.9 ± 9.8 a	49.4 ± 6.9 a	54.1 ± 8.3 a	3.4 ± 1.0 a	1.2 ± 0.6 a
I	80	5.4 ± 0.4 a	4.1 ± 0.2 a	5.8 ± 0.2 a	10.7 ± 0.4 a	26.0 ± 0.9 a	52.9 ± 4.3 a	31.8 ± 6.8 a	23.3 ± 4.2 a	28.2 ± 4.6 a	1.5 ± 0.8 c	1.0 ± 0.5 a
C	40	5.4 ± 0.3 a	4.6 ± 0.2 a	5.4 ± 0.2 a	10.2 ± 0.2 a	25.7 ± 0.8 a	70.8 ± 5.1 a	53.2 ± 7.9 a	42.5 ± 5.8 a	48.3 ± 6.5 a	3.0 ± 0.9 b	1.3 ± 0.6 a
C	80	5.4 ± 0.3 a	4.3 ± 0.2 a	5.2 ± 0.2 a	9.4 ± 0.3 b	24.2 ± 0.6 a	45.0 ± 5.6 a	27.6 ± 5.3 a	17.8 ± 3.7 a	23.5 ± 4.3 a	1.5 ± 0.8 c	1.0 ± 0.5 a

Loc – locations, I – inland, C – coastal, Hum – humidity, * – days from second instar of nymphs to adults. Mean ± standard error (SE). Mean followed by different letters in the same column are significantly different at p = 0.05, using IRRISTAT program for Windows 5.0. Means were compared by the method of least significant differences (LSD) at 5% level.

Appendix 4. The interaction effects of locations and humidity regimes on body weight and reproductive performance and egg hatchability of *Nezara viridula* (L.)

Loc	Hum (%)	Mean weight (g) ± SE of adult <i>N. viridula</i>			Mating frequency (times)	Pre-mating period (days)	Pre-oviposition period (days)	Egg-masses/female	Egg-mass size (eggs/egg mass)	Fecundity (Eggs/female)	% of egg mass hatched	Egg hatch-ability (%)
		Female	Male	Adults (F & M)								
I	40	0.14 ± .01 a	0.12 ± .00 a	0.13 ± .01 a	2.7 ± 0.9 a	25.4 ± 7.2 a	30.7 ± 9.4 a	0.5 ± 0.2 a	27.9 ± 9.4 a	30.3 ± 13.3 a	38.3 ± 12.4 a	7.1 ± 2.7 a
I	80	0.13 ± .01 a	0.11 ± .00 a	0.12 ± .01 a	1.3 ± 0.6 a	9.4 ± 4.7 a	12.9 ± 6.8 a	0.3 ± 0.2 a	14.3 ± 7.5 c	16.9 ± 9.7 a	25.0 ± 13.1 a	22.3 ± 11.7 a
C	40	0.14 ± .01 a	0.11 ± .01 a	0.12 ± .01 a	2.7 ± 1.0 a	19.8 ± 6.8 a	33.7 ± 11.1 a	1.0 ± 0.4 a	23.1 ± 7.6 b	52.5 ± 22.3 a	35.5 ± 11.7 a	6.2 ± 2.1 a
C	80	0.14 ± .01 a	0.11 ± .01 a	0.12 ± .01 a	1.9 ± 0.7 a	16.1 ± 4.4 a	12.0 ± 6.5 a	0.5 ± 0.3 a	15.0 ± 7.8 c	32.1 ± 17.4 a	25.0 ± 13.1 a	21.9 ± 11.4 a

Loc – locations, I – inland, C – coastal, Hum – humidity, Mean ± standard error (SE). Mean followed by different letters in the same column are significantly different at p = 0.05, using IRRISTAT program for Windows 5.0. Means were compared by the method of least significant differences (LSD) at 5% level.

Appendix 5. The interaction effects of locations, temperature and humidity regimes on nymphal duration, survival, adult longevity and egg hatching duration. Bolded results are significant differences between locations within the same climatic condition.

Loc	Tem (°C)	Hum (%)	Mean duration ± SE of nymphal stages (days)				Nymphal duration* (days)	Nymphal survival (%)	Mean longevity ± SE of adult <i>N. viridula</i>		Adults (F & M)	Incubation period (days)	1 st instar duration (days)
			2nd	3rd	4th	5th			Female	Male			
I	25	40	6.9 ± 0.4 ab	5.5 ± 0.2 a	6.6 ± 0.3 a	11.5 ± 0.6 a	30.5 ± 1.2 a	71.7 ± 12.0 a	90.3 ± 11.8 a	72.0 ± 8.2 a	84.8 ± 9.0 a	6.9 ± 0.1 ab	4.9 ± 0.1 a
I	25	80	7.2 ± 0.1 a	5.2 ± 0.3 a	6.3 ± 0.5 a	11.1 ± 0.7 a	29.9 ± 1.4 a	66.7 ± 6.0 a	45.8 ± 5.2 a	43.0 ± 7.7 cd	44.7 ± 5.9 a	6.0 ± 0.1 c	4.0 ± 0.1 a
I	30	40	5.5 ± 0.3 cde	4.3 ± 0.2 a	5.5 ± 0.1 a	10.7 ± 0.4 a	26.1 ± 0.7 a	78.3 ± 7.3 a	70.1 ± 8.3 a	67.9 ± 4.1 ab	69.3 ± 2.6 a	6.8 ± 0.2 b	-
I	30	80	5.0 ± 0.1 fg	4.3 ± 0.4 a	6.0 ± 0.1 a	10.7 ± 0.5 a	26.0 ± 0.3 a	50.0 ± 2.9 a	46.5 ± 21.1 a	13.3 ± 3.1 gh	31.9 ± 10.8 a	-	-
I	33	40	4.8 ± 0.4 efgh	3.9 ± 0.2 a	5.0 ± 0.2 a	9.8 ± 0.8 a	23.5 ± 0.8 a	58.3 ± 1.7 a	53.9 ± 16.3 a	37.3 ± 6.8 cde	44.8 ± 10.6 a	-	-
I	33	80	5.3 ± 0.3 de	3.3 ± 0.2 a	5.6 ± 0.3 a	11.5 ± 1.0 a	25.7 ± 1.4 a	43.3 ± 11.7 a	27.2 ± 5.7 a	24.7 ± 4.1 efg	26.5 ± 2.8 a	-	-
I	36	40	4.9 ± 0.7 efgh	3.9 ± 0.2 a	5.5 ± 1.6 a	9.5 ± 0.3 a	23.9 ± 0.8 a	45.0 ± 12.6 a	13.4 ± 6.6 a	20.3 ± 1.9 fgh	17.6 ± 3.5 a	-	-
I	36	80	4.0 ± 0.1 h	3.9 ± 0.2 a	5.2 ± 0.2 a	9.3 ± 0.1 a	22.4 ± 0.0 a	51.7 ± 8.8 a	7.7 ± 0.6 a	12.2 ± 1.4 gh	9.7 ± 0.1 a	-	-
C	25	40	6.8 ± 0.3 ab	5.9 ± 0.2 a	6.4 ± 0.1 a	10.7 ± 0.3 a	29.8 ± 0.7 a	80.0 ± 12.6 a	61.9 ± 3.1 a	39.3 ± 4.2 cde	59.5 ± 6.2 a	7.1 ± 0.2 a	4.4 ± 0.1 a
C	25	80	6.4 ± 0.3 abc	5.0 ± 0.2 a	5.5 ± 0.4 a	9.9 ± 0.6 a	26.9 ± 1.5 a	66.7 ± 6.0 a	45.0 ± 11.2 a	31.0 ± 5.9 def	38.3 ± 8.6 a	6.0 ± 0.2 c	3.8 ± 0.2 a
C	30	40	5.2 ± 0.1 def	4.5 ± 0.1 a	5.4 ± 0.1 a	10.2 ± 0.1 a	25.3 ± 0.1 a	78.3 ± 8.8 a	85.4 ± 5.0 a	60.1 ± 14.2 ab	66.9 ± 11.2 a	5.0 ± 0.0 d	1.0 ± 1.0 a
C	30	80	6.0 ± 0.7 bed	4.4 ± 0.3 a	5.1 ± 0.1 a	8.8 ± 0.7 a	24.3 ± 0.3 a	46.7 ± 8.8 a	35.4 ± 6.5 a	25.3 ± 4.3 efg	31.8 ± 4.6 a	-	-
C	33	40	5.5 ± 0.1 cde	4.1 ± 0.2 a	5.0 ± 0.3 a	9.5 ± 0.7 a	24.1 ± 1.0 a	65.0 ± 2.9 a	50.2 ± 5.2 a	51.6 ± 1.9 bc	49.7 ± 1.6 a	-	-
C	33	80	4.7 ± 0.2 efgh	3.8 ± 0.0 a	4.7 ± 0.4 a	10.1 ± 0.3 a	23.2 ± 0.5 a	35.0 ± 12.6 a	24.5 ± 3.5 a	7.3 ± 2.8 h	16.2 ± 3.8 a	-	-
C	36	40	4.1 ± 0.1 gh	4.0 ± 0.2 a	5.0 ± 0.1 a	10.5 ± 0.2 a	23.6 ± 0.4 a	60.0 ± 13.2 a	15.4 ± 6.1 a	19.2 ± 6.5 fgh	17.1 ± 5.8 a	-	-
C	36	80	4.3 ± 0.1 fgh	4.1 ± 0.5 a	5.4 ± 0.1 a	8.8 ± 0.5 a	22.6 ± 0.7 a	31.7 ± 6.0 a	5.6 ± 2.4 a	7.7 ± 3.2 h	7.5 ± 2.0 a	-	-

Loc – locations, I – inland, C – coastal, Tem – temperature, Hum – humidity, F – female, M – male, * – days from second instar of nymphs to adults. Mean ± standard error (SE). Mean followed by different letters in the same column are significantly different at p = 0.05, using IRRISTAT program for Windows 5.0. Means were compared by the method of least significant differences (LSD) at 5% level.

Appendix 6. The interaction effects of locations, temperature and humidity regimes on body weight, reproductive performance and egg hatchability of *Nezara viridula* (L.)

Loc	Tem (°C)	Hum (%)	Mean weight (g) ± SE of adult <i>N. viridula</i>			Mating frequency (times)	Pre-mating period (days)	Pre-oviposition period (days)	Egg-masses/female	Egg-mass size (eggs/egg mass)	Fecundity (Eggs/female)	% of egg mass hatched	Egg hatchability (%)
			Female	Male	Adults (F & M)								
I	25	40	0.17 ± .01 a	0.13 ± .01 a	0.15 ± .01 a	6.3 ± 1.2 a	31.6 ± 3.0 a	53.6 ± 2.1 c	1.3 ± 0.2 a	73.3 ± 9.5 a	96.0 ± 25.7 a	73.1 ± 4.6 a	19.5 ± 2.3 a
I	25	80	0.16 ± .01 a	0.13 ± .01 a	0.14 ± .01 a	4.7 ± 0.3 a	20.2 ± 1.3 a	51.6 ± 1.7 c	1.2 ± 0.4 a	57.3 ± 1.6 a	67.7 ± 18.8 a	100.0 ± 0.0 a	89.3 ± 3.9 a
I	30	40	0.15 ± .01 a	0.13 ± .00 a	0.14 ± .01 a	4.0 ± 2.0 a	48.6 ± 6.5 a	69.1 ± 1.7 b	0.7 ± 0.3 a	38.3 ± 1.7 a	25.2 ± 12.7 a	80.0 ± 20.0 a	8.7 ± 4.7 a
I	30	80	0.14 ± .01 a	0.11 ± .01 a	0.13 ± .01 a	-	-	-	-	-	-	-	-
I	33	40	0.14 ± .00 a	0.12 ± .00 a	0.13 ± .00 a	0.3 ± 0.3 a	21.3 ± 21.3 a	-	-	-	-	-	-
I	33	80	0.12 ± .01 a	0.10 ± .01 a	0.11 ± .01 a	0.3 ± 0.3 a	17.3 ± 17.3 a	-	-	-	-	-	-
I	36	40	0.12 ± .01 a	0.10 ± .01 a	0.11 ± .00 a	-	-	-	-	-	-	-	-
I	36	80	0.11 ± .00 a	0.10 ± .01 a	0.10 ± .01 a	-	-	-	-	-	-	-	-
C	25	40	0.15 ± .01 a	0.12 ± .00 a	0.14 ± .01 a	8.3 ± 0.3 a	25.6 ± 2.6 a	47.0 ± 1.6 c	2.7 ± 0.6 a	60.1 ± 1.1 a	163.6 ± 34.1 a	57.2 ± 9.7 a	14.5 ± 3.0 a
C	25	80	0.16 ± .00 a	0.12 ± .01 a	0.14 ± .00 a	5.3 ± 1.7 a	25.7 ± 4.7 a	48.2 ± 8.3 c	2.1 ± 0.4 a	59.9 ± 1.5 a	128.3 ± 22.0 a	100.0 ± 0.0 a	87.5 ± 2.6 a
C	30	40	0.16 ± .00 a	0.12 ± .01 a	0.14 ± .00 a	2.3 ± 0.7 a	53.6 ± 6.4 a	87.8 ± 2.4 a	1.3 ± 0.8 a	32.5 ± 2.5 a	46.2 ± 29.3 a	84.8 ± 15.2 a	10.4 ± 2.3 a
C	30	80	0.14 ± .01 a	0.12 ± .01 a	0.13 ± .01 a	2.0 ± 0.6 a	31.5 ± 3.8 a	-	-	-	-	-	-
C	33	40	0.13 ± .00 a	0.12 ± .00 a	0.12 ± .00 a	-	-	-	-	-	-	-	-
C	33	80	0.14 ± .00 a	0.09 ± .02 a	0.12 ± .01 a	0.3 ± 0.3 a	7.3 ± 7.3 a	-	-	-	-	-	-
C	36	40	0.10 ± .00 a	0.09 ± .01 a	0.10 ± .01 a	-	-	-	-	-	-	-	-
C	36	80	0.12 ± .01 a	0.10 ± .00 a	0.11 ± .01 a	-	-	-	-	-	-	-	-

Loc – locations, I – inland, C – coastal, Tem – temperature, Hum – humidity. Mean ± standard error (SE). Mean followed by different letters in the same column are significantly different at p = 0.05, using IRRISTAT program for Windows 5.0. Means were compared by the method of least significant differences (LSD) at 5% level