The potential impact of climate change on *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and its parasitoid, *Trichopoda giacomelii* Blanchard (Diptera: Tachinidae) in Cambodia and Australia: Ecological, behavioural and physiological assessments.

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(Photos by: Gerhard Körtner 2011)

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Declaration

I certify that the substance of my thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that, to the best of my knowledge, any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

[redacted]

Signature

Chanthy Pol

Front cover: Top left: Adults of male and female *Nezara viridula*

Top right: Adult of parasitoid fly, *Trichopoda giacomellii*

Bottom left: 5th instar nymph of *N. viridula*

Bottom right: Adult of *N. viridula* attached with *T. giacomellii* eggs

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Table of Contents

List of abbreviations

ACIAR	Australian Centre for International Agricultural Research
ASEM	Agricultural System and Economic Management
BS	Beat sheet
AusAID	Australian Agency for International Development
BTB	Battambang province
CARDI	Cambodian Agricultura Research and Development Institutre
CO_2	Carbon dioxide
CTL	Critical thermal limits
CT_{Max}	Critical thermal maximum
CT_{Min}	Critical thermal minimum
$CT_{MinOnset}$	Critical thermal minimum onset
NSW DPI	NSW Department of Primary Industries
IPM	Integrated Pest Management
GVB	Green vegetable bug (*Nezara viridula*)
Hsps	Heat shock proteins (hsps)
KCM	Kampong Cham province
JAF	John Allwright Fellowship
K	Thermal constant (*K*)
L:D	Light:dark ratio
$LiClH_2O$	Lithium chloride
LLT	Lower lethal temperature
LT	Lethal temperature

$MgCl_2.6H_2O$	Magnesium chloride
NSW	New South Wales
R5	Reproductive growth stage of soybean: beginning seed-early podfill
R6	Reproductive growth stage of soybean: full seed-late podfill
RCH	Rapid cold hardening
RH	Relative humidity (%)
SD	Standard deviation
SE	Standard error
SN	Sweep netting
ULT	Upper lethal temperature
T_0	Threshold temperature (T_0)

Thesis summary

Nezara viridula (L.) (Hemiptera: Pentatomidae) is a cosmopolitan, polyphagous heteropteran insect that causes economic damage to many crop species worldwide. Despite several interventions attempts, *N. viridula* has remained a significant pest in certain regions of Cambodia and eastern Australia, particularly those where soybean and nut crops are cultivated. Recently, *Trichopoda giacomellii* Blanchard (Diptera: Tachinidae), a species native to Argentina was established in Australia as an effective biological control agent for *N. viridula* in one instance. However, with predicted climate change, the range of the pest could expand or change and the relationship between the pest and the parasitoid could also change in response to altered temperature and precipitation regimes. Changed climatic conditions could affect the pest status and geographic range of *N. viridula* as well as the efficacy of the parasitoid. Climate change will potentially affect the interactions between factors such as temperature, humidity, light, food, and the wellbeing of the pest and the parasitoid and the relationship between the two.

The aims of this study were (i) to evaluate the sampling methods of arthropods on soybean crops in Cambodia, in terms of accuracy of the use of the two main sampling methods: sweep netting and beat sheeting; (ii) to measure the effect of changed temperature and moisture regimes on *N. viridula* and *T. giacomellii* life cycles in populations from contrasting climatic regimes (Breeza and Grafton); (iii) to measure the effect of changed temperature and moisture regimes on the ability of the parasitoids to parasitize *N. viridula*; and (iv) to investigate the various physiological variables of *N. viridula* under the stress of temperature.

A survey was conducted to evaluate two sampling methods, sweep netting and beat sheeting to estimate population size of arthropods in soybean crops in Cambodia. Sweep netting captured a wider diversity of insect orders compared to estimates from beat sheeting. However, the beat sheeting caught significantly higher numbers of Acari. There were no significant differences between sweep net and beat sheet for capture of Mantodae, Odonata and Strepsiptera. The rate of new species accumulating in each sampling method was relatively low for beat sheeting compared to sweep netting for Hemiptera and Hymenoptera. The estimated numbers of Hemipteran and Hymenopteran species caught by beat sheeting and sweep netting together are expected to be 1.5 times higher than the actual number. For individual methods, the estimation of Chao-1 of Hemiptera and Hymenoptera caught by sweep netting were much higher compared to beat sheeting. Between locations and sampling methods, the assemblages of Hemiptera and Hymenoptera were significantly different. Between samples of beat sheeting for Hemiptera and Hymenoptera, the dispersion (or variation) was significantly higher compared to sweep netting, but there were no differences in dispersion between locations.

Both collection methods had advantages and disadvantages for use in the Cambodian soybean farm context. The beat sheet performed best in dry field conditions, upright plants and was more accurate and easy to relate to area (number of insects per square meter) and caught *N. viridula* more effectively compared to sweep net. However, the sweep net method can be used universally in row or non row crops and wet or dry field conditions. Sweep netting caught higher numbers of arthropods compared to beat sheeting, but the sweep net results are difficult to convert to densities on an area basis.

The developmental time of nymphal stages and the percentage of nymphal survival from 2nd instar to adults of *N. viridula* decreased significantly with increasing temperature and humidity regimes. The longevity of *N. viridula* adults declined with increasing temperature or humidity regimes and the females lived longer than the males. High temperatures (30, 33 and 36ºC) or high humidity (80% RH) reduced reproductive performance and capacity of *N. viridula* compared to low temperature (25ºC) and low humidity (40%). However, high humidity significantly improved egg hatchability.

Interactions of temperature and humidity regimes significantly affected incubation period of *N. viridula*. Adult longevity was greater at low humidity and shortened with increasing temperature regimes. Mating frequency, pre-mating period and egg-mass size of *N. viridula* significantly decreased with increasing humidity. At the same humidity regime with different temperatures (25, 30, 33, and 36ºC), mating frequency and egg-mass size decreased with increasing humidity regimes. Percentage of egg-mass hatching and egg hatchability increased significantly at the high humidity regime at 25ºC. Interactions of location and temperature significantly affected incubation period, pre-mating period, pre-oviposition period, egg-mass per female, and fecundity of *N. viridula* from Breeza and Grafton. Interactions of location and humidity were significant for incubation period and egg-mass size of *N. viridula*. Interactions of location, temperature and humidity regimes were significant for incubation period and pre-oviposition period.

Developmental time of *T. giacomellii* significantly decreased with increasing temperature. *Trichopoda giacomellii* could not complete full development at 36ºC. The percentage of pupal mortality of *T. giacomellii* at 25ºC was significantly lower compared to 30ºC, 33ºC and 36ºC. Pupal weight at 25ºC was significantly heavier compared to pupal weight at 30ºC, 33ºC and 36ºC. Percentage of pupation and weight of pupae of *T. giacomellii* at low humidity (40% RH) were significantly higher compared to high humidity.

Interactions of temperature and humidity regimes had a significant effect on the duration of the larval period of populations of *T. giacomellii*. At 33ºC (across all humidity treatments), the larval period of Grafton populations was significantly longer than the larval period of Breeza populations. The temperature and humidity regimes and interactions of temperature and humidity had no effect on parasitisation or total numbers of eggs laid by *T. giacomellii* females. There were no significant differences between parasitism of male and female *N. viridula* in all climate conditions.

At 20ºC, under a constant photoperiod of L:D 10:14 h., diapause was readily induced in Breeza and Grafton *N. viridula* populations. The critical temperature for diapause of Breeza and Grafton *N. viridula* was 15ºC, since at this temperature regime diapause was induced in more than 50% of the population. At 10ºC, 100% of the *N. viridula* population entered diapause. The diapause induction of Breeza *N. viridula* reared at 20ºC and 15ºC with low and high humidity was higher than for Grafton *N. viridula* reared at 20ºC and 15ºC with low and high humidity. At 20 and 15ºC under constant photoperiod of L:D 10:14 h., the larval and pupal periods of *T. giacomellii* was significantly longer compared to those at 25ºC, suggesting that larvae or pupae may enter diapause under the low temperature regimes with short daylength. Overall, these results suggested that *T. giacomelii* would develop synchrony with the host, *N. viridula* under these changed climatic conditions.

The upper and lower lethal temperatures for *N. viridula* at two contrasting climate locations (Breeza and Grafton, NSW Australia) were 40.3ºC and -8.0ºC under the stress of high and low temperatures with 20% survival. The survival of *N. viridula* increased after acclimation at high temperature for seven days. In contrast, when acclimated at 10 and 15ºC, the survival of Breeza and Grafton *N. viridula* declined. The 25ºC control reared *N. viridula* adults had a mean $CT_{MinOnset}$ (cold stupor) of 1.3 ± 2.1ºC and a mean CT_{Max} (heat coma) of 45.9 ± 0.9ºC. CT_{Max} and $CT_{MinOnset}$ of Breeza and Grafton *N. viridula* populations did not differ for acclimation temperatures. These results suggest that short-term temperature acclimation is more important than the provenance of collection (and thus the temperatures that populations have been exposed to over multiple generations). CT_{Max} of *N. viridula* did not differ between 25ºC reared (control) and high temperature acclimated groups. $CT_{MinOnset}$ of 10ºC-acclimated *N. viridula* was significantly lower than that of *N. viridula* from the 25ºC control by 1.1ºC. This suggests that *N. viridula* species are more tolerant of low temperature when acclimated at 10ºC.

The results indicated that humidity is important for insect survival, development and reproduction, but not so critical a factor as is temperature, and that each species has an optimum which may be different for various stages of the life cycle. In *N. viridula* the nymphs develop faster at low humidity (40 ± 10% RH), but the eggs of *N. viridula* and pupae of *T. giacomellii* develop more rapidly at high humidity (80 ± 10% RH). Optimum temperature for development of *N. viridula* and its parasitoid, *T. giacomellii* was likely to be 25ºC, since all developmental stages developed successfully with the low humidity of 40 ± 10% RH for *N. viridula* and high humidity (80 ± 10% RH) for pupae of *T. giacomellii*. No significant differences were recorded for developmental time of Breeza and Grafton *N. viridula* and *T. giacomellii* populations. At 20ºC with the constant photoperiod of L:D 10:14 h., *N. viridula* and *T. giacomellii* readily entered the diapause stage. The critical temperature for diapause induction of *N. viridula* was 15ºC. *T. giacomelii* would develop synchrony with their host, *N. viridula* under this changed climate condition. The upper and lower lethal temperatures did not differ between the two populations of *N. viridula* collected at Breeza and Grafton. *Nezara viridula* responds more strongly to extreme high temperature as it showed increased survival at upper lethal temperature when acclimated at high temperature for seven days.

Further research is warranted for: determination of upper and lower lethal temperatures of nymphal stages of *N. viridula*; the recovery response after exposure to

extreme temperature; rapid cold hardening (RCH); desiccation resistance of *N. viridula* for adults and nymphal stages; and desiccation resistance of pupae of *T. giacomellii*.