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# Comparisons of the thermal physiology of water hyacinth biological control agents: predicting establishment and distribution pre- and post-release

Bronwen May* & Julie Coetzee

*Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa*

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## Abstract

Investigations into the thermal physiology of weed biological control agents may elucidate reasons for establishment failure following release. Such studies have shown that the success of water hyacinth biological control in South Africa remains variable in the high-lying interior Highveld region, because the control agents are restricted to establishment and development due to extreme winter conditions. To determine the importance of thermal physiology studies, both pre- and post-release, this study compared the known thermal requirements of *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae) released in 1996, with those of an agent released in 1990, *Niphograpta albiguttalis* (Warren) (Lepidoptera: Pyralidae) and a candidate agent, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), which is currently under consideration for release. The lower developmental threshold ($t_o$) and rate of development (K) were determined for *N. albiguttalis* and *M. scutellaris*, using a reduced axis regression, and incorporated into a degree-day model which compared the number of generations that *E. catarinensis*, *N. albiguttalis*, and *M. scutellaris* are capable of producing annually at any given site in South Africa. The degree-day models predicted that *N. albiguttalis* ($K = 439.43$, $t_o = 9.866$) can complete 4–11 generations per year, whereas *M. scutellaris* ($K = 502.96$, $t_o = 11.458$) can only complete 0–10 generations per year, compared with *E. catarinensis* ($K = 342$, $t_o = 10.3$) which is predicted to complete 3–14 generations per year. This suggests that the candidate agent, *M. scutellaris*, will not fare better in establishment than the other two agents that have been released in the Highveld, and that it may not be worth releasing an agent with higher thermal requirements than the agents that already occur in these high-lying areas. Thermal physiology studies conducted prior to release are important tools in biological control programmes, particularly those in resource-limited countries, to prevent wasting efforts in getting an agent established.

## Introduction

The successful establishment and spread of biological control agents are necessary for effective control of invasive weeds, and often depends on the similarity of the climate conditions in the area of origin and the area of introduction (Samways et al., 1999), as temperature is one of the principal abiotic factors that influences an animal's development rate (Campbell et al., 1974; Chown & Nicholson, 2004), distribution (Klok & Chown, 1997), and abundance (van der Merwe et al., 1997). Numerous biological control agents have failed to establish in areas of introduction due to climate incompatibility (Crawley, 1986; McClay, 1996; Byrne et al., 2002, 2003) and this could have been ameliorated by investigating the thermal physiological requirements of the biological control agents and conducting climate matching studies prior to their release.

Studies on the thermal requirements of agents are generally conducted post-release to provide possible explanations for failed establishment of biological control agents (Coetzee et al., 2007a). However, by this stage, failure of establishment of the agents represents a waste of time and funding (Byrne et al., 2003), particularly in resource-limited countries. Globally, weed biological control practitioners do not conduct thermal physiology studies prior to

*Correspondence: E-mail: g07m2502@campus.ru.ac.za

 

an agent's release because they are time consuming and it is perceived to be of greater importance to release as many host-specific agents, with the hope that at least one of the agents will establish and bring about successful control (McEvoy & Coombs, 1999). Nonetheless, although climate matching studies are time consuming, they are not tedious projects and do not require extensive funding. Furthermore, 'pre-release' thermal physiology studies enable the selection of appropriate and well-adapted candidate species, thereby reducing expenditure in trying to get agents established following release.

In South Africa, thermal requirement and climate matching studies have been equally neglected within weed biological control programmes as they have been worldwide. The biological control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms. (Pontederiaceae), South Africa's most problematic aquatic weed (Hill & Olckers, 2000; Coetzee et al., 2011), has been variable, despite the release of seven biological control agents: six arthropods and one pathogen, more than anywhere else in the world (Coetzee et al., 2011). Most of the worst infestations occur in the Highveld, the high-lying interior plateau (Hill & Olckers, 2000; Coetzee et al., 2007a) (Figure 1). Extreme winter temperatures, ca. 3–4 months of frost, and eutrophic waters typify the conditions of this region, reducing the ability of the agents to significantly impact water hyacinth infestations (Hill & Cilliers, 1999; Hill & Olckers, 2000; Coetzee et al., 2007b). Due to the lack of pre-release thermal requirement investigations, the majority of the biological agents released for water hyacinth control in South Africa are fundamentally adapted to low-altitude warm climates and none has been selected for high-altitude cooler climates. The biological control agents fail to persist in the Highveld, as they cannot resist cold stress during winter, mainly due to their warm tropical origin (Amazon Basin, South America), reducing their ability to increase to numbers high enough for effective control, particularly in the early warmer months, when nutrient-enriched waters allow water hyacinth to recover rapidly from winter die-back (Hill & Olckers, 2000). The densities of the weed at this time are too great for the biological control agents to make a significant reduction in the

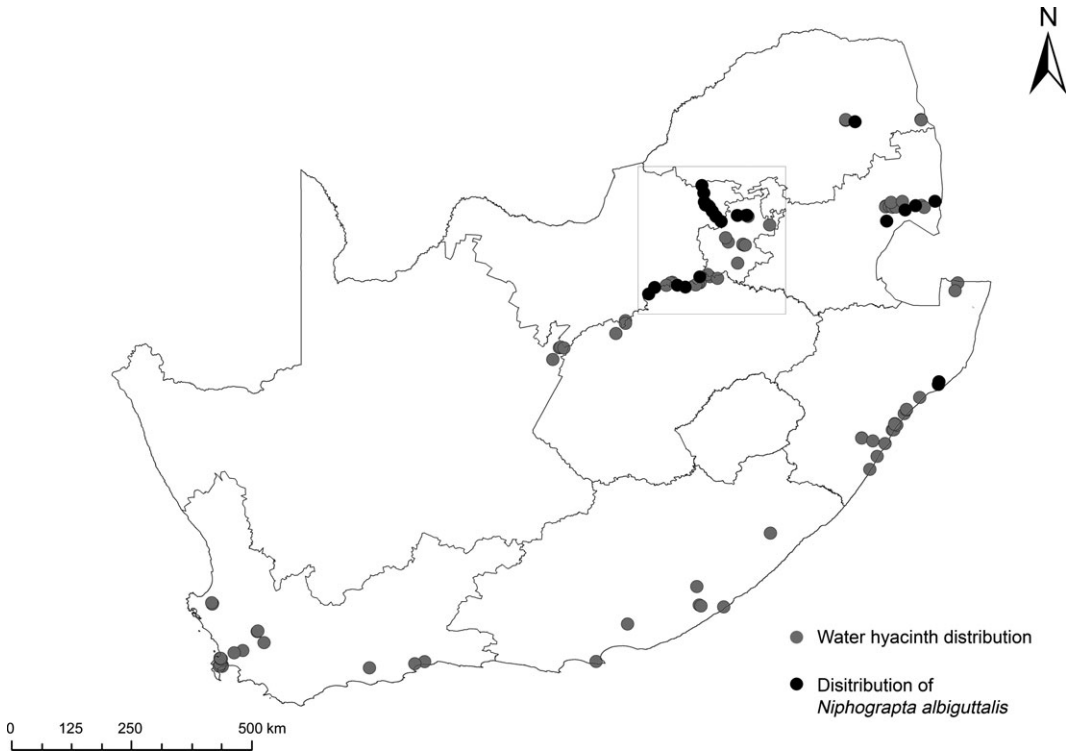


**Figure 1** The distribution of water hyacinth and establishment sites of the moth, *Niphograpta albiguttalis* across South Africa. The most serious weed infestations occur in the Highveld along the Vaal (Free State and North West Provinces) and Crocodile Rivers (northern Gauteng and North West Provinces) (indicated by the square), where water systems are typified by eutrophic conditions and cold winters. The moth has established at sites along the Vaal and Crocodile River.

infestation (Coetzee et al., 2007a). Undoubtedly, new approaches are needed to keep water hyacinth densities in the Highveld at levels of minimal impact.

Even though low winter temperatures are assumed to be a major factor reducing the effectiveness of water hyacinth biological control, the thermal requirements of only one agent, *Eccritotarsus catarinensis* (Carvalho) (Heteroptera: Miridae), have been investigated. *Eccritotarsus catarinensis* is a leaf-feeding bug, released in South Africa in 1996 for the control of water hyacinth (Coetzee et al., 2007a). Since then, it has established at various sites across South Africa, but its distribution remains patchy, particularly in the higher altitude areas (Coetzee et al., 2007a). Coetzee et al. (2007a) showed that the development and distribution of *E. catarinensis* are limited during the cold winters in the Highveld due to its inability to develop sufficiently rapidly, reducing its efficacy as a biological control agent. This study was the first attempt at determining the climate suitability and potential distribution of an agent for the control of water hyacinth in South Africa, to explain its limited establishment.

Even though the degree-day model for *E. catarinensis* was conducted post-release, the model provides a basis to which other models for other water hyacinth biological control agents can be compared, whether they are determined pre- or post-release. The thermal physiology of the other five water hyacinth biological control agents have yet to be investigated and their potential distribution determined, to aid in explaining their establishment failures and successes. *Niphograpta albiguttalis* (Warren) (Lepidoptera: Pyralidae) is a petiole-boring moth and was first released into South Africa in the 1990s (Julien et al., 2001). Since then, few post-release evaluation studies have been conducted to determine its current distribution and establishment. Numerous references based on field and laboratory observations have been made which suggest that it is cold hardy. It is suggested to have a wide climatic tolerance (Cilliers, 1991) and the larvae are able to survive temperatures as low as 4 °C in winter (Julien et al., 2001). In South Africa, the moth has established along the Vaal and Crocodile Rivers, in the Highveld region, and other typically cold regions of the escarpment, as well as along the subtropical KwaZulu-Natal coast, near Richards Bay (Figure 1) (JA Coetzee, pers. obs.). The effectiveness of this moth as a biological control agent has possibly been undervalued (Julien, 2001) and due to its potential cold hardiness, promoting its establishment in the Highveld should be prioritized – if it is proven to be climatically suited – before releasing a new agent.

Additional climatically suited biological control agents are being investigated for potential release into South Africa to control water hyacinth (Cordo, 1999). Field surveys within the Amazon catchment (mainly in Peru, South America) identified a number of new species specific to water hyacinth, with potential as biological control agents (Cordo, 1999). One such control agent, *Megamelus scutellaris* (Berg) (Hemiptera: Delphacidae), a sap-sucking planthopper, has subsequently been selected as a candidate for release in the cooler high-altitude regions of the Highveld, because of its cooler Argentine and Peruvian origins (Tipping et al., 2008). It is currently under investigation in quarantine at Rhodes University, South Africa. Although *M. scutellaris* has not yet been released in South Africa, the Agricultural Research Service (ARS) of the USDA released the agent in the USA in 2010 and its establishment is being monitored (Center & Tipping, 2010). Determining the physiological range in which it is able to overwinter in South Africa would justify its release in the Highveld, or at any other suitable water hyacinth sites.

Therefore, the aim of this study was to develop a degree-day model for *N. albiguttalis* and *M. scutellaris* in South Africa, by investigating for both species the thermal parameters lower developmental threshold ($t_o$) and rate of development (K). Developing degree-day models for these two agents allowed for prediction of their potential distributions through calculation of the number of generations that they are capable of producing annually and over the colder winter months at any given site in South Africa, particularly in the Highveld. The predicted distributions and thermal requirements of *N. albiguttalis* and *M. scutellaris* with those of *E. catarinensis* and the known distribution of water hyacinth in South Africa will serve as a comparison between pre- and post-release thermal physiology studies, and will allow for the selection of climatically suitable agents for release at appropriate water hyacinth sites.

## Materials and methods

### Development rates

Individuals used for the thermal physiology experiments with *N. albiguttalis* and *M. scutellaris* were collected from cultures at Rhodes University, Grahamstown, South Africa (33°18′48.08″S, 26°31′05.93″E). The *N. albiguttalis* cultures were initiated in 2009 from individuals collected from Bon Accord Dam, South Africa (25°37′48.52″S, 28°11′23.25″E), where the moth has established since its release in 1989. The *M. scutellaris* cultures were initiated in 2007 from individuals sent from the USDA, ARS Invasive Plant Research Lab, Fort Lauderdale (26°05′04.53″S, 80°14′23.57″E).

Thermal physiology experiments with *N. albiguttalis* and *M. scutellaris* were run in 2009 and 2010, respectively. *Niphograpta albiguttalis* and *M. scutellaris* were reared

from egg to adult in five controlled environment rooms, each set at a different experimental temperature. The constant experimental temperatures ranged from 18 to 30 °C, with a L12:D12 photoperiod. Nine-litre buckets were set up with two water hyacinth plants in each. Mesh sleeves which allowed light and air to penetrate were placed over the buckets and securely fastened with elastic bands to ensure that the insects did not escape. Plants from 10 buckets were each inoculated with 10 *N. abiguttalis* adults, while plants from another 10 buckets were each inoculated with 20 *M. scutellaris* adults, at a 1:1 sex ratio. The adults were left to mate and oviposit for 24 h; this ensured that the eggs were at most a day old. For each temperature treatment and insect species, two buckets were simultaneously placed in each of the respective controlled environment rooms and temperature trials were run concurrently.

Water hyacinth leaves were observed daily for newly hatched neonate *N. albiguttalis* larvae and *M. scutellaris* nymphs. The number of days that the eggs took to hatch was recorded. Fifty neonate *N. albiguttalis* larvae and 30 *M. scutellaris* nymphs were then removed from the water hyacinth plants from each temperature treatment, and placed individually in poly-top vials (30 ml) with air-tight lids. *Niphograpta albiguttalis* larvae have a feeding preference for bulbous water hyacinth growth forms (Julien et al., 2001); therefore a sectioned bulbous water hyacinth petiole was placed into the vials. *Megamelus scutellaris* nymphs simply feed on the sap from the leaf (Tipping et al., 2008); therefore water hyacinth leaf discs (3 $cm^2$) were used for *M. scutellaris*. A strip of filter paper was added to absorb excess moisture droplets transpired from the water hyacinth tissue to prevent any moisture build-up that might trap and cause the death of larvae or nymphs. Petioles and leaves were replaced daily. Thermochron iButton data loggers (Climastats, Environmental Monitoring software, version 4; Fairbridge Technologies, Johannesburg, South Africa) recorded the actual temperatures experienced in the controlled environment rooms. Thermochron iButtons were also placed in vials with a water hyacinth petiole or leaf as operative thermometers to obtain accurate measures of the temperature within the closed environment. Daily observations were made of the life stages; the number of days taken to reach each instar was recorded, as verified by the measurement of *N. albiguttalis* head capsule width (Center et al., 1982) and the exuviae left behind between each instar of *M. scutellaris*. The total number of days for each individual in each temperature treatment to reach the adult stage was determined.

The development rates, developmental thresholds, and consequent emergence to eclosion degree-day requirements were calculated from these data. The relationship between temperature and developmental rate is not linear, especially at the lower and upper temperature thresholds. For this reason, the reduced major axis regression method was utilized. Ikemoto & Takai (2000) found this method to produce more accurate thermal parameters than the linear regression model proposed by Campbell et al. (1974), as they are formulated from more precise detections of the upper and lower critical temperatures. The thermal requirements and the slopes of the regression lines of *N. albiguttalis* and *M. scutellaris* were compared with those of *E. catarinensis*, by performing an ANCOVA and Tukey's honestly significant difference (HSD) post-hoc test in STATISTICA 9 (Statsoft, Tulsa, OK, USA) (Shi et al., 2010).

#### Lower developmental threshold ($t_o$) and the rate of development (K)

Temperature and time are used in degree-day modelling to predict the number of generations that an agent can complete at a given site, in a given year (Coetzee et al., 2007a). The number of days to complete full development (D) was plotted against the temperature multiplied by the number of days to complete full development (DT). The lower developmental threshold ($t_o$) and the thermal constant K were calculated using the equation obtained from the reduced major axis method, where $t_o$ is determined from t and k (y-intercept) represents K: $DT = k - tD$. The thermal constant K is the number of degree days of development above the developmental threshold $t_o$, the temperature at which zero development occurs. There is no measure of error associated with the reduced major axis method, as the parameters K and $t_o$ are drawn straight from the line parameters (Ikemoto & Takai, 2000).

The thermal parameters obtained from the reduced major axis method were used to develop degree-day models for *N. albiguttalis* and *M. scutellaris*. The effect of microclimate temperatures is suggested to be essential in predicting the distribution and establishment of an insect, as it presents a more accurate estimate of the actual temperatures that the insect may experience under field conditions (Coetzee et al., 2007a). Therefore, 2 years (2005–2006) of mean daily minimum and maximum temperatures of water hyacinth canopies from 15 long-term water hyacinth monitoring sites across the country (Byrne et al., 2010) were used to calculate the accumulated degree days at these sites (Figure 2), using the equation: $K = \Sigma[(T_{max} + T_{min})/2 - t]$. The mean annual degree days accumulated for each of the 15 sites was then determined which in turn gave the number of generations that *N. albiguttalis* and *M. scutellaris* are capable of producing at each site per year, by dividing the mean accumulated

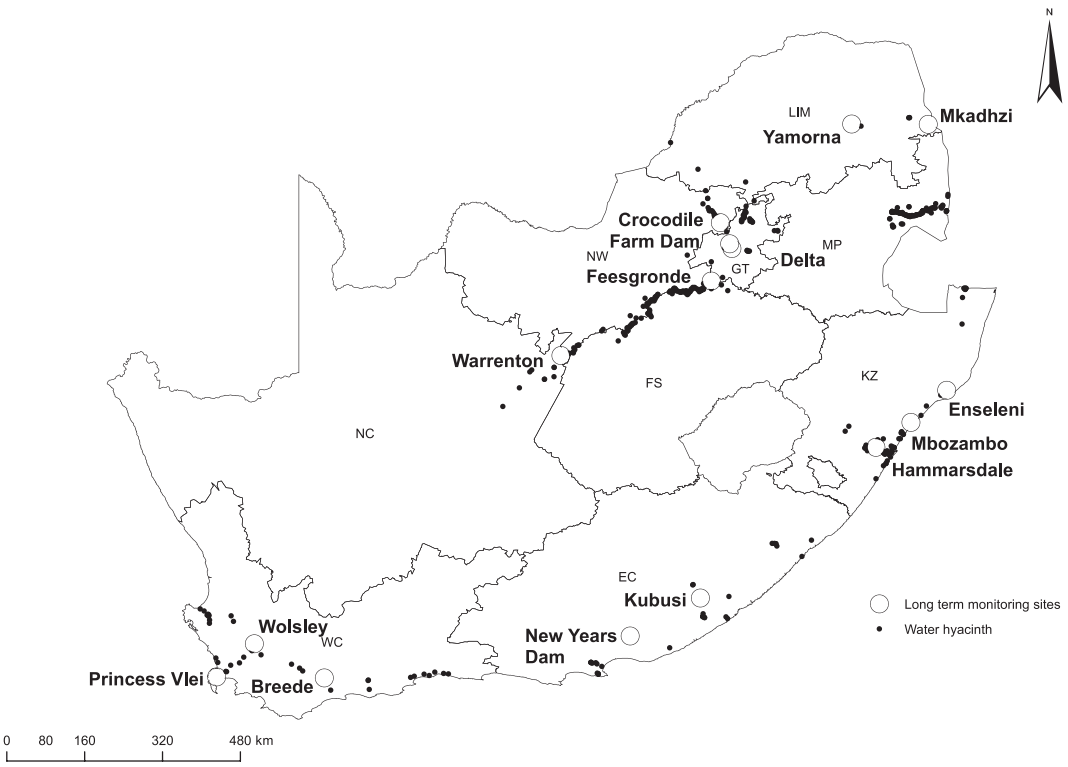


**Figure 2** The 15 long-term water hyacinth monitoring sites across South Africa, which have 2 years worth of mean daily minima and maxima water hyacinth canopy air temperatures.

degree days by the number of degree days of development (K) for both insect species.

Maps were created using ArcGIS version 10 (ESRI, Midrand, South Africa), indicating the number of generations that *N. albiguttalis* and *M. scutellaris* potentially complete in a year at these 15 sites. The number of generations that *N. albiguttalis* and *M. scutellaris* potentially produce over the winter months, from May to August, was also calculated and displayed graphically in a map of South Africa. Using a one-way ANOVA and a Tukey's HSD post-hoc test in STATISTICA 9, the numbers of generations that *N. albiguttalis*, *M. scutellaris*, and *E. catarinensis* are capable of producing at the 15 sites per year and during the winter months were compared.

## Results

### Development rates

*Niphograpta albiguttalis* completed its life cycle from emergence to eclosion at all temperature treatments (Table 1); however, completion of the full life cycle of *M. scutellaris* did not occur at all temperatures as nymphs did not develop beyond the first instar at 30 °C (Table 2). Both *N. albiguttalis* and *M. scutellaris* exhibited a typical relationship between development and temperature. A slower rate of development was observed at lower temperatures and a faster rate at higher temperatures. At the lowest temperature, 16.7 °C, *N. albiguttalis* larvae completed development in 62.7 ± 1.2 days (mean ± SEM, n = 3) and at 30 °C the larvae completed development in 22.1 ± 0.83 (n = 8) days (Table 1). At both the highest and lowest temperatures, there was a high mortality rate for *N. albiguttalis* individuals (68 and 85.7% mortality respectively). *Megamelus scutellaris* completed development in 65.6 ± 0.52 days (n = 10) at the lowest temperature, 19 °C, and in 39.4 ± 1.38 days (n = 12) at the highest temperature, 27 °C (Table 2).

### Lower developmental threshold ($t_o$) and the thermal constant (K)

The equations and thermal parameters obtained from the reduced major axis model for *N. albiguttalis* and *M. scutellaris* are compared in Table 3, along with the parameters for *E. catarinensis* (Coetzee et al., 2007a). The development of *N. albiguttalis* and *M. scutellaris,* from emergence to eclosion, required 439.43 and 502.96 degree days (K), above a threshold of 9.87 and 11.46 °C ($t_o$) respectively.

There was a significant difference between the degree-day models for all three insects (ANCOVA: $F_{2,42}$ = 28.25,

**Table 1** Mean (± SEM) number of days taken for *Niphograpta albiguttalis* to reach a life stage and to complete full development at 18–30 °C treatment

| Life stage | 18 °C (16.73 °C[1]) | 22 °C | 24 °C (22.65 °C[1]) | 27 °C (25.37 °C[1]) | 30 °C |
|---|---|---|---|---|---|
| Instar 1 – 2 | 8.43 ± 0.20 (21) | 5.39 ± 0.13 (28) | 5.04 ± 0.15 (26) | 4.72 ± 0.17 (25) | 3.6 ± 0.19 (25) |
| Instar 2 – 3 | 7.11 ± 0.15 (19) | 3.96 ± 0.11 (24) | 3.88 ± 0.12 (25) | 3.61 ± 0.10 (23) | 3.23 ± 0.15 (22) |
| Instar 3 – 4 | 7 ± 0.24 (17) | 4.09 ± 0.15 (22) | 3.84 ± 0.15 (25) | 3.53 ± 0.12 (23) | 3.23 ± 0.16 (22) |
| Instar 4 – 5 | 7.06 ± 0.78 (16) | 4.18 ± 0.14 (22) | 4.48 ± 0.25 (25) | 4.04 ± 0.11 (23) | 3.48 ± 0.18 (21) |
| Instar 5 – 6 | 5.82 ± 0.77 (11) | 5.45 ± 0.47 (16) | 5.92 ± 0.36 (12) | 7.4 ± 0.24 (5) | No 6th instar[2] |
| Instar 6 – pupation | 16 ± 0.37 (6) | 8.81 ± 0.70 (11) | 6.7 ± 0.63 (22) | 8.55 ± 0.59 (20) | 6.64 ± 0.43 (13) |
| Pupation – eclosion | 21 ± 0 (3) | 10.46 ± 0.24 (13) | 11.785 ± 0.72 (14) | 9.75 ± 0.43 (12) | 7.88 ± 0.23 (8) |
| Total (1st instar – adult) | 62.67 ± 1.2 | 34.54 ± 0.48 | 33.5 ± 0.70 | 30.5 ± 0.54 | 22.13 ± 0.83 |

Sample sizes (number of *N. albiguttalis* individuals) are indicated in parentheses.
[1]The actual temperature recorded within the controlled environment room.
[2]Individuals did not develop an extra (6th) instar.

**Table 2** Mean (± SEM) number of days taken for *Megamelus scutellaris* to reach a life stage and to complete full development at 19–30 °C treatment

| Life stage | 19 °C | 22 °C | 25 °C | 27 °C | 30 °C |
|---|---|---|---|---|---|
| Egg – 1st instar | 25.41 ± 0.076 (42) | 17 ± 0 (51) | 13 ± 0 (40) | 10 ± 0 (40) | 9 ± 0 (49) |
| Instar 1 – 2 | 9.46 ± 1.80 (24) | 6.28 ± 0.084 (47) | 4.54 ± 0.081 (39) | 4.61 ± 0.12 (38) | No data[1] |
| Instar 2 – 3 | 7.57 ± 0.15 (23) | 4.91 ± 0.077 (45) | 3.59 ± 0.079 (39) | 5.31 ± 0.28 (35) | – |
| Instar 3 – 4 | 6.95 ± 0.85 (19) | 5.45 ± 0.098 (42) | 4.62 ± 0.090 (37) | 7.17 ± 0.61 (29) | – |
| Instar 4 – 5 | 7.57 ± 1.28 (14) | 5.61 ± 0.14 (41) | 4.43 ± 0.091 (37) | 7.29 ± 1.95 (21) | – |
| Instar 5 – adult | 8.3 ± 1.15 (10) | 6.62 ± 0.15 (37) | 6.15 ± 0.12 (36) | 8.25 ± 1.82 (12) | – |
| Total (egg – adult) | 65.6 ± 0.52 | 45.71 ± 0.27 | 36.25 ± 0.20 | 39.42 ± 1.38 | – |

Sample sizes (number of *M. scutellaris* individuals) are indicated in parentheses.
[1]No individuals at 30 °C survived passed the 1st instar.

**Table 3** The thermal parameters K and $t_0$ obtained from the reduced major axis method for *Niphograpta albiguttalis*, *Megamelus scutellaris*, and *Eccritotarsus catarinensis*

| Parameter | *Niphograpta albiguttalis* | *Megamelus scutellaris* | *Eccritotarsus catarinensis*[1] |
|---|---|---|---|
| Equation | DT = 9.8657x + 439.43 | DT = 11.458x + 502.96 | DT = 10.39x + 341.75 |
| K | 439.43 | 502.96 | 342 |
| $t_o$ | 9.866 | 11.458 | 10.3 |
| Regression coefficient | $F_1 = 283.35$, $R^2 = 0.855$, $P<0.05$ | $F_1 = 375.68$, $R^2 = 0.800$, $P<0.05$ | $R^2 = 0.931$, $P<0.05$ |

[1]Data taken from Coetzee et al. (2007a).

$P<0.05$). *Eccritotarsus catarinensis* (Ec) and *N. albiguttalis* (Na) have similar thermal requirements (Na: $t_o$ = 9.87 °C, Ec: $t_o$ = 10.3 °C); however, *E. catarinensis* has a lower thermal constant, suggesting shorter generation times than *N. albiguttalis* (Na: K = 439.43, Ec: K = 342). *Megamelus scutellaris* has significantly higher thermal requirements (K = 502.96, $t_o$ = 11.46 °C), than *N. albiguttalis* and *E. catarinensis*. The lower developmental threshold of *M. scutellaris* is fairly high in terms of cold climate compatibility. This may have important implications for the potential cold hardiness of *M. scutellaris* in the Highveld.

#### Degree-day accumulation

The number of generations that *N. albiguttalis* and *M. scutellaris* are capable of producing in 1 year at any given site was estimated from the thermal parameters obtained from the reduced major axis method and the microclimate canopy temperature data of 15 sites (Figures 3 and 4). The same procedure was used to determine

the number of generations that *N. albiguttalis* and *M. scutellaris* are capable of producing over the winter months, May to August (Figures 3 and 4). The moth is capable of producing a minimum of four and maximum of 11 generations per year at any locality in South Africa (Figure 3), whereas *M. scutellaris* can only complete 0–10 generations per year at any locality (Figure 4). The degree-day model for *E. catarinensis* predicts 3–14 generations per year at any locality in South Africa (Coetzee et al., 2007a).

*Niphograpta albiguttalis* was predicted to produce 5–8 generations in a year in the high-altitude region of the escarpment, particularly the Highveld. However, over the winter period, *N. albiguttalis* was estimated to produce 1.8 generations at Feesgronde, along the Vaal River, 1.0 generation at Delta Park, Johannesburg, and 1.1 generations along the Crocodile River (Figure 3). *Eccritotarsus catarinensis* was predicted to produce similar numbers of generations in this area over the winter period. Conversely, *M. scutellaris* was predicted to produce fewer generations in the Highveld during winter, with 0.5 generations produced at Delta Park and Crocodile River, 0.1 at Farm Dam, and 1.2 generations at Feesgronde (Figure 4). At Enseleni, a warmer subtropical site, *N. albiguttalis* and *M. scutellaris* are capable of producing 2.6 and 1.9 generations, respectively, over the winter months. These values indicate that the low winter temperatures of the Highveld limit development and distribution of *N. albiguttalis* and *M. scutellaris* and that their development is restricted to the summer months.

There was no significant difference between the predicted number of generations produced by *N. albiguttalis* and *E. catarinensis* in any given year, but *M. scutellaris* was predicted to produce fewer generations than both the moth and the mirid ($F_{2,42} = 11.91$ $P<0.05$). The same result is observed for the winter months ($F_{2,42} = 6.03$, $P<0.05$).

## Discussion

Hill & Olckers (2000) proposed that low winter temperatures limit the successful establishment of biological control agents of water hyacinth in South Africa. Thermal physiology studies, preferably conducted pre-release, allow biological control practitioners to identify these mismatches and to select and release climatically compatible biological control agents into suitable areas.

The results of this study suggest that although low temperatures will limit *N. albiguttali*s development over

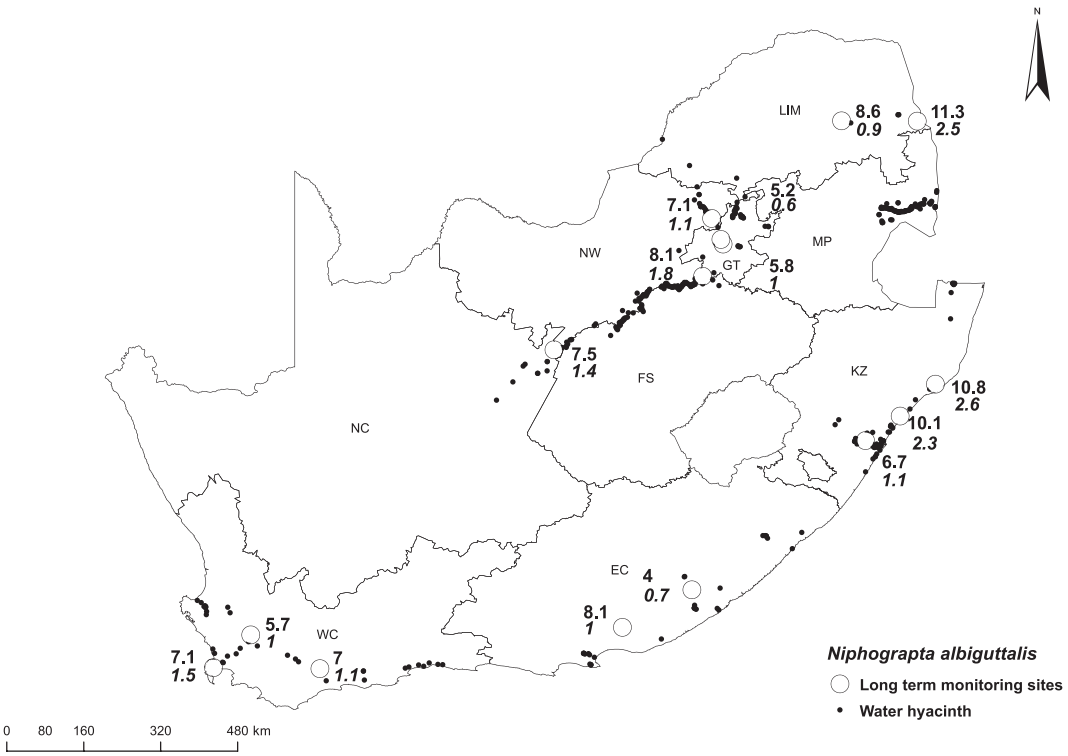


**Figure 3** Number of generations that *Niphograpta albiguttalis* is capable of producing per year (top value) and during the winter months (bottom value) at 15 sites in South Africa, estimated from the reduced major axis model and 2 years of daily water hyacinth canopy temperatures.

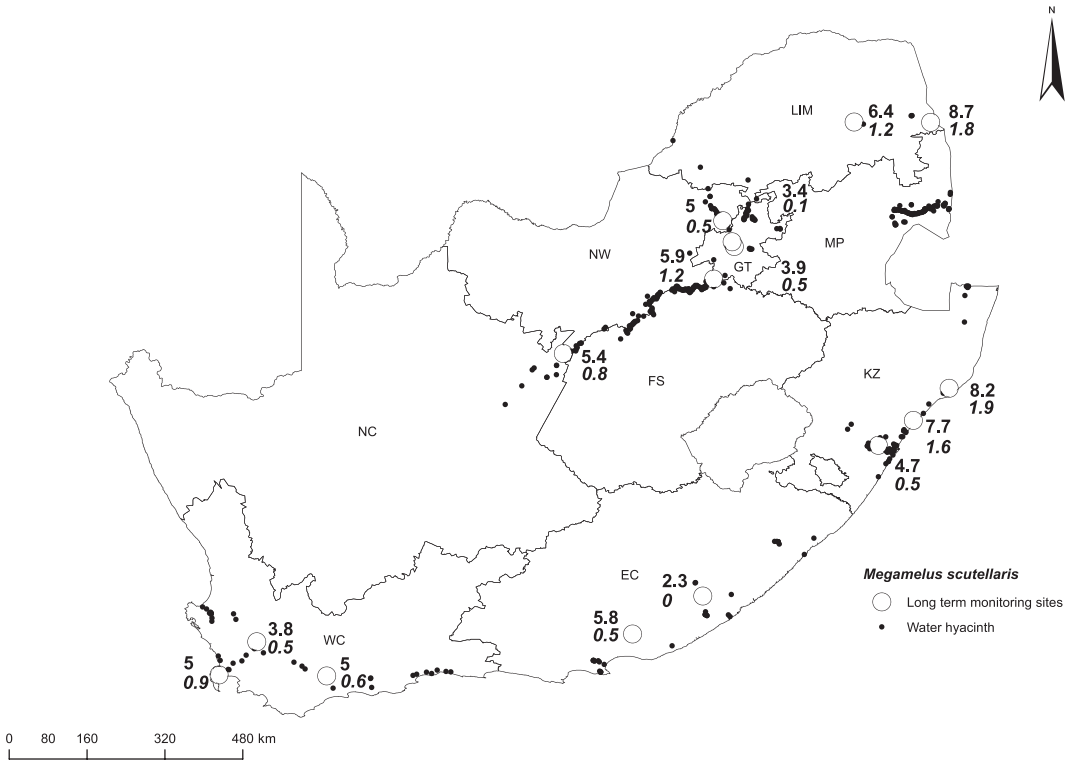


**Figure 4** Number of generations that *Megamelus scutellaris* is capable of producing per year (top value), and during the winter months (bottom value) at 15 sites in South Africa, estimated from the reduced major axis model and 2 years of daily water hyacinth canopy temperatures.

the cold winters in the Highveld, the moth can persist and complete development over this period. It is predicted to complete at least one generation during the winter months, indicating its persistence over this period which is evidenced by it having established populations along the Vaal and Crocodile Rivers (JA Coetzee, pers. obs.). The moth's predicted establishment in the Highveld is further supported by the similarity of the climate conditions occurring in its area of origin and the area of introduction. The original distribution of *N. albiguttalis* in South America extends into tropical and temperate regions (Julien et al., 2001), whereas the Highveld is typified by a temperate climate (Byrne et al., 2010).

Such climatic conditions would undoubtedly have a more severe impact on *M. scutellaris* if it were released into the Highveld, due to its high thermal requirements, as suggested by the insect's thermal physiology. Although *M. scutellaris* was selected as a candidate agent due to its cooler Argentinean origin, it may not be worth exhausting resources into the release of this agent in the Highveld. *Megamelus scutellaris* is not capable of producing any generations over the cold winters in the Highveld and is therefore climatically incompatible with the low winter temperatures experienced in the high-lying areas in South Africa. This raises the question of whether it is worth releasing an agent that has higher thermal requirements than agents already established in the Highveld.

The numbers of generations that *E. catarinensis* and *N. albiguttalis* are capable of producing per year at each of the 15 sites were not significantly different. This suggests that these two agents have evolved under similar climatic conditions in South America and have similar thermal requirements. Essentially, *N. albiguttalis* and *E. catarinensis* are better adapted to the cooler South African climate than *M. scutellaris*, therefore the investment of resources into the release of *M. scutellaris* into the Highveld should not be promoted. However, *M. scutellaris* is very damaging (Center & Tipping, 2010), and has potential to be a good agent in areas where negligible water hyacinth control has been obtained, and the climate conditions are favourable. *Megamelus scutellaris* is capable of producing between 2.3 and 8.7 generations per year at any of the subtropical and coastal sites, and its release in these areas should be encouraged. The field release of *M. scutellaris* would supplement the damage caused by the other water hyacinth biological control agents, particularly the two *Neochetina* species, in the subtropical and coastal regions.

Fundamentally, in comparison to the thermal physiology of *M. scutellaris*, the moth is the most suitable agent for release in the Highveld. Therefore, there is potential for the development of an augmentative approach with improved release techniques. Center & Durden (1981) facilitated the release of *N. albiguttalis* in Florida, USA, and successful establishment was observed at sites where young, bulbous plants were dominant, the preferred growth form of *N. albiguttalis* (Center & Durden, 1981; Julien et al., 2001). In addition, Center & Durden (1981) observed seasonal differences and the suitability of different life stages for establishment. At one particular site, establishment was best when first instars were released, as opposed to adults, at a particular time of year. The release efforts noted by Center & Durden (1981) emphasize that climate incompatibility is not the only contributing factor to failed establishment of biological control agents; the manner and technique in which biological control agents are released into the field is another factor limiting the establishment of biological agents (Hill & Olckers, 2000; McEvoy & Coombs, 2000). Other factors include nutrient enrichment and the overall hydrology of the water systems (Hill & Olckers, 2000).

In South Africa, the worst water hyacinth infestations occur in nutrient-enriched water systems in high-altitude sites characterized by cold conditions (Hill & Cilliers, 1999). In these conditions, the weed takes on a colonizing bulbous form in early spring to recover from winter die-back (Hill & Olckers, 2000). Eventually, due to eutrophication and low insect populations, infestations become dense and the plants take on a tall slender form in early summer (B May, pers. obs.). The development of a combined augmentative approach to incorporate both *E. catarinensis* and *N. albiguttalis* for the control of water hyacinth in the Highveld would be appropriate, as both these agents have similar thermal requirements. The mirid can be mass-reared and released in early summer, shortly after the release of *N. albiguttalis*, which favours the colonizing bulbous form (Julien et al., 2001), and because the mirid has a short generation time (Hill et al., 1999), *E. catarinensis* populations can increase rapidly to attack the taller, slender water hyacinth forms which form in early summer. In addition, *N. albiguttalis* has not been released throughout South Africa (Figure 1) (B May, pers. obs.), and there are various sites across South Africa where negligible water hyacinth control has been obtained. The redistribution of *N. albiguttalis* into these sites should be encouraged, especially as the moth is extremely damaging and the development of an augmentative approach for the cooler areas is conceivable.

In conclusion, post-release evaluations are essential tools and cannot be neglected once a biological control programme has been initiated or a new agent has been released. It is the responsibility of biological control practitioners to ensure their science has been implemented as effectively as possible. Determining the reasons for failed establishment can lend itself useful to future research and to the improvement of augmentative control strategies. It goes without saying that climate modelling and thermal physiology studies are equally important. This study serves to highlight that the release of agents should only be considered once thermal physiology studies and climate modelling have been conducted to ensure potential success in the cold regions, and that post-release evaluations are required to justify the release of new candidate species and the continued expenditure of resources.

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