Life history parameters and predation capacities of *Nesidiocoris volucer*: a new biological control agent for tomato crop

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Abstract

Whiteflies are one of the major pests of tomato under greenhouses, and their control partly relies on biocontrol strategies. Among those biocontrol agents, parasitoids or predators are widely used. However, the introduction of a biocontrol agent in a new area is not trivial. For that reason, we investigated the use of a tropical native mirid, *Nesidiocoris volucer* (Hemiptera: Miridae), for the biological control of whiteflies among other insect pests on tomato crops under greenhouses in the subtropical island of La Réunion, France. *Nesidiocoris volucer* life history traits and plant injury were examined. Nymphs developed and survived between 15 and 30°C and required on average 49.41 days at 15°C and on average 10.50 days at 30°C to develop (nymph survival >94%). At 25°C, each female produced on average 65 eggs. *Nesidiocoris volucer* was able to feed on several prey species, but performed better on whiteflies than on spider mites or thrips. No *N. volucer* feeding injury was observed on tomato. *Nesidiocoris volucer* has also been found in tropical countries of Africa, and we believe that the data presented on this natural enemy could be of great importance for the biocontrol of whiteflies in tropical areas.

Introduction

The use of invertebrate biological control agents to reduce the population of another organism considered as a pest has been widely adopted, and proved its efficiency, as an environmentally safe pest management method (Gurr and Wratten, 2012; Van Lenteren et al., 2018). Mirids (Hemiptera: Miridae) have been studied and three main genus *Macrolophus*, *Dicypsy* and *Nesidiocoris* have been used as biological control agents (Van Lenteren, 2012; Van Lenteren et al., 2018). These mirids are often considered both pests of major crops and natural predators of diverse invertebrates such as whitefly species, aphids, leafminers, thrips, mite, lepidopteran eggs, small larvae, etc. (Wheeler, 2001). Studies of these mirids have mostly focused on four species: *Dicypsy taminanni* Wagner, *Dicypsy hesperus* Knight, *Macrolophus pygmaeus* Rambur and *Nesidiocoris tenuis* Reuter (Castañé et al., 2011). Except for *D. taminanni*, these species are currently being commercially mass reared as biological control agents (Van Lenteren, 2012). *Nesidiocoris tenuis* is used to control whiteflies (Hemiptera: Aleyrodidae), leafminers (Lepidoptera: Gelechiidae), thrips (Thysanoptera: Thripidae) and spider mites (Arachnida: Tetanychidae) mainly on tomato but also on eggplant and sweet pepper (Riudavets and Castañé, 1998; Ryckewaert and Alauzet, 2002; Urbaneja et al., 2005, 2009). However, the beneficial status of *N. tenuis* is controversial (Pérez-Hedo and Urbaneja, 2016) because this mirid becomes a plant feeder that causes significant crop damage when prey numbers are low (Arnó et al., 2006, 2010; Sanchez and Lacasa, 2008; Sanchez, 2009).

In Reunion, which is a subtropical island in the Indian Ocean, tomato is the main vegetable crop and is mostly produced in greenhouses, where whiteflies and spider mites are the main pests (Lange and Bronson, 1981). In addition to causing direct damage to plants, whiteflies vector plant viruses (Polston et al., 2014). To help control whiteflies, farmers can release the parasitoids *Encarsia formosa* and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae), both of which are mass reared by the company La Coccinelle© in Reunion (Saint-Pierre). Predators of whiteflies have not yet been reared in Reunion; however, farmers reported increasing problems to control whiteflies with only those parasitoids. Based on the lack of predators as biocontrol agents of tomato pests, we investigated potential indigenous candidates using literature records and direct observations. Among the different candidates, records of a predatory mirid, *Nesidiocoris volucer*, closely related to *N. tenuis*, described on the island in 1902 (Kirkaldy, 1902) caught our attention. Indeed, *N. volucer* was detected preying on the whitelly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) (authors’ personal communication),...
suggesting its potential as a biological control agent. This mirid had also been reported in other tropical countries of Africa such as Tanzania (Kilimanjaro), Cap Verde, Mozambique, Uganda and Sudan (Schuh, 2002–2013) and most recently Niger (Garba et al., 2020) showing its pantropical distribution.

Before considering an organism for use in biological control, thorough investigation of its biology and ecology is needed. The development and maintenance of sufficient numbers of the agent to protect the crop can be affected by temperature, food availability and many other factors (Bale et al., 2009; Gurr and Wratten, 2012). In this study, we investigated the life history and predation capacities of the indigenous *N. volucer*, as a potential new biocontrol agent of herbivorous pests under greenhouse conditions. We conducted experiments under controlled conditions to evaluate the influence of temperature on *N. volucer* development, survival of immature stages, and fecundity when feeding on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). We then determined the predation capacity of *N. volucer* on the following greenhouse pests: *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), *Tetranychus* sp. (Acari: Tetranychidae), *Thrips parvispinus* Karny (Thysanoptera: Thripidae) and *T. vaporariorum*. Finally, we determined whether *N. volucer* injures tomato plants.

**Materials and methods**

**Insects used in this study**

**Tobacco whitefly**

A laboratory population of the exotic invasive whitefly *B. tabaci* MEAM1 (formerly named B biotype) established from field-collected individuals in 2001 (Delatte et al., 2005) on cotton (*Gossypium* sp.) was used. This MEAM1 colony is kept in several ventilated Plexiglas containers (60 × 40 × 60 cm) in a climate (temperature and humidity controlled) room at 25°C, 70% relative humidity (RH), and a 16:8 (L:D) h photoperiod. For experiments, leaves with 4th–5th instar nymphs were collected and examined with a dissecting microscope to remove any drops of honeydew that are naturally produced by *B. tabaci* instars.

**Greenhouse whitefly**

A colony of *T. vaporariorum* initiated (from field-collected individuals) in 2010 on tobacco plants (*Nicotiana tabacum* L.) in the La Coccinelle© greenhouses was used. Leaves with 4th–5th instar nymphs were collected for the different experiments.

**Spider mite**

Spider mite specimens of *Tetranychus* sp. group desertorum (probably *T. evansi* species, the only described species from this group in Reunion) were collected from a natural population *Solanum lycopersicum* (L.) in a greenhouse (ARMEFLHOR, Saint Pierre). To determine the species, DNA was extracted from four individuals and a portion of the gene encoding cytochrome oxidase I (COI) was used for DNA barcoding. Paratypes in ethanol-preserved tubes are available in the insect collection of CIRAD, UMR PVBM in Saint-Pierre, La Réunion, voucher no. MTEN00002_0201. Larvae and adults were transferred to young cotton leaves obtained from insect-free plants that had been grown in a climate room at 25°C, 70% RH and a 16:8 (L:D) h photoperiod.

**Predatory bug**

A wild population of the predatory bug *N. volucer* was collected in 2010 from weeds in Saint Paul, La Réunion and further maintained on a *T. vaporariorum* colony in the La Coccinelle© facility. Since then, the *N. volucer* population has been maintained on *T. vaporariorum* on tobacco in a greenhouse at the company. Specimens of this *N. volucer* population were examined by a mirid specialist (JC Streito, INRA, CBGP, France) who confirmed their identity. In addition, DNA was extracted from four specimens, and a portion of the gene that encodes COI was used for DNA barcoding of the *N. volucer* population. Paratypes are available in the insect collection of CIRAD, UMR PVBM in Saint-Pierre, La Réunion, vouchers no. MATI00012_0101 and MATI00035_0101; GenBank accession numbers KT201360 and KT201350. All *N. volucer* specimens used in this study originated from the same population at the La Coccinelle© facility.

**Data analysis**

All statistical analyses were performed with the statistical program R 4.1.1 (R Development Core Team, 2021). Different statistical approaches were used, as detailed below in each experimental section: Fisher’s exact tests or χ² tests, pairwise Wilcoxon rank-sum tests, and the generalized linear mixed model [GLMM, package lme4 (Bates et al., 2014)], with deviance tests on fixed effects (based on a χ² test) and, if necessary, Tukey’s all pair comparison tests [package multcomp (Hothorn et al., 2008)]. A Bonferroni-like correction was applied for pairwise tests. The *P*-values of significance level are indicated for all tests.

**Experimental design**

**Climate chambers description**

All experiments were conducted in five climate chambers (Sanyo, MLR-350) that enabled control of temperature. Each of the five climate chambers was set with a temperature according to the experimental design (15, 20, 25, 30 and 35°C). A data logger (HOBO U12-013, Onset®, USA) was inserted in each chamber to confirm temperature and RH during all experiments.

**Plastic cups design used throughout the study**

All of the laboratory experiments used plastic cups (48 mm diameter, 23 mm high). Each cup contained a leaf disc of the tested plant, which was placed on a 5 mm layer of agar (1%). A sharp round plastic shape of 46 mm of diameter was used to obtain each leaf disc. Each cup also had a lid with a ventilation hole covered with fine-mesh gauze.

**Development of nymphs**

The developmental times for *N. volucer* nymphs were studied at 15, 20, 25, 30 and 35°C, with 76 ± 9% RH and a 12:12 (L:D) h photoperiod (Sanyo, MLR-350). For doing so, 120 *N. volucer*
adults (60 males and 60 females) were collected from the La Coccinelle© greenhouse and transferred to mesh cages (50 × 50 × 50 cm) containing a 30 cm-tall tobacco plant, on which they mated and laid eggs. Adults were removed 4 h later, and the tobacco plant was transferred to a climate chamber (Sanyo, MLR-350) at 25°C and with 60 ± 10% RH and a 12:12 (L:D) h photoperiod. Hatched nymphs were collected daily and individually caged in plastic cups (48 mm diameter, 23 mm high) and maintained in plastic cups described above with a tobacco leaf disc placed on a 5 mm layer of agar (1%) sprinkled with an E. kuehniella (Biobest©) eggs provided ad libitum at each of the five temperatures. Development and survival of nymphs were recorded daily, and the sex of newly emerged adults was determined. For each temperature, 61–72 individual nymphs were monitored, as a whole the development of 330 nymphs were individually followed (tables 1 and 2). This set up allowed us to follow individually each of nymphs until their adult stage.

Effects of sex and rearing temperature on the duration of N. volucer nymph development were analysed by a pairwise Wilcoxon rank test with a Bonferroni-like correction. The effect of rearing temperature on overall survival was analysed with a Fisher’s exact test on a contingency table containing the total number of dead nymphs and living adults at each temperature. For each temperature, the comparison of instar survival was done by a Fisher’s exact test using a contingency table containing the number of dead and living nymphs at each instar. Differences in the sex ratio between rearing temperatures were tested with a χ² test.

### Development of eggs

To determine the developmental time of eggs, eggs were obtained using the same protocol that was used to obtain hatched nymphs but the tobacco plants with eggs were directly transferred into a climate chamber at each of the five temperatures. Ephesia kuehniella eggs were sprinkled on the leaves, and the newly hatched nymphs were counted daily. For each temperature, 49–150 eggs were studied (tables 1 and 2). A pairwise Wilcoxon rank test was done with a Bonferroni-like correction to compare egg developmental durations between the five rearing temperatures.

### Fecundity

The fecundity of 33 N. volucer females was investigated at 25 ± 1°C and with 89 ± 6% RH and a 16:8 (L:D) h photoperiod (in a climate chamber Sanyo, MLR-350). To obtain newly emerged adult to conduct this experiment, over hundreds of 4th–5th-instar nymphs of N. volucer were collected from the greenhouse rearing and maintained in plastic cups described above with a tobacco leaf disc placed on a 5 mm layer of agar (1%) sprinkled with an ad libitum quantity of E. kuehniella eggs. Newly emerged adults were collected each day for use in this experiment. Then, one female with two males, newly emerged, were transferred to a ventilated cylinder plastic cage (88 mm diameter, 255 mm high) with a mature tomato leaflet sprinkled with E. kuehniella eggs. In total, 33 cups, containing one female and two males were used. All leaflets were kept at 25°C (in a climate chamber Sanyo, MLR-350) and were inspected daily. In order to always keep fresh leaves for egg laying, adults were transferred to a new ventilated cylinder plastic cage with a mature tomato leaflet sprinkled with E. kuehniella eggs twice each week. Dead males were replaced until the female died. All leaflets kept at 25°C (for each female cylinder plastic cage) were inspected daily for hatched nymphs during 2 weeks after adult removal. Whenever hatched nymphs were observed, they were removed from the leaflets and counted to assess fecundity for each of the 33 females. This set up allowed us to follow individually the fecundity of each of the female until their death. Nesidiocoris volucer fecundity (as indicated by the number of hatched nymphs) was statistically analysed by a GLMM with a Poisson distribution, with weeks as a fixed effect and with each individual female as a random effect. A pairwise

### Table 1. Mean (SEM) development time (in days) for eggs and nymphs of Nesidiocoris volucer at various temperatures (in °C)

| Temp (°C) | N   | Hatching time | Sex | N | 1st instar | 2nd instar | 3rd instar | 4th instar | 5th instar | 1st instar to adult* |
|----------|-----|---------------|-----|---|------------|------------|------------|------------|------------|---------------------|
| 15       | 150 | 27.59 (0.06)  |     | 70| 9.78 (0.14)| 7.81 (0.08)| 8.26 (0.07)| 9.57 (0.09)| 14.06 (0.08)| 49.41 (0.25)       |
|          |     |               |     | F | 9.79 (0.26)| 7.67 (0.11)| 8.18 (0.09)| 9.36 (0.1) | 13.88 (0.11)| 48.88 (0.4)        |
|          |     |               |     | M | 9.73 (0.14)| 7.94 (0.11)| 8.33 (0.11)| 9.70 (0.14)| 14.24 (0.12)| 49.94 (0.29)       |
| 20       | 51  | 13.94 (0.10)  |     | 67| 4.72 (0.08)| 3.67 (0.06)| 3.79 (0.06)| 4.58 (0.06)| 7.43 (0.07)| 24.19 (0.15)       |
|          |     |               |     | F | 4.74 (0.14)| 3.75 (0.08)| 3.71 (0.1) | 4.49 (0.09)| 7.17 (0.08)| 23.71 (0.22)       |
|          |     |               |     | M | 4.69 (0.08)| 3.6 (0.09) | 3.88 (0.07)| 4.69 (0.08)| 7.72 (0.10)| 24.72 (0.18)       |
| 25       | 49  | 8.18 (0.06)   |     | 60| 3.12 (0.06)| 2.17 (0.05)| 2.31 (0.06)| 2.56 (0.07)| 3.98 (0.06)| 14.12 (0.11)       |
|          |     |               |     | F | 3.14 (0.08)| 2.28 (0.1) | 2.21 (0.08)| 2.69 (0.09)| 3.79 (0.08)| 14.10 (0.18)       |
|          |     |               |     | M | 3.07 (0.08)| 2.07 (0.05)| 2.4 (0.09) | 2.43 (0.09)| 4.17 (0.07)| 14.13 (0.14)       |
| 30       | 87  | 6.14 (0.04)   |     | 72| 2.07 (0.03)| 1.93 (0.03)| 1.49 (0.06)| 1.92 (0.03)| 3.09 (0.04)| 10.50 (0.07)       |
|          |     |               |     | F | 2.03 (0.03)| 1.91 (0.05)| 1.5 (0.09) | 1.91 (0.05)| 3.0 (0.04) | 10.35 (0.09)       |
|          |     |               |     | M | 2.11 (0.05)| 1.94 (0.04)| 1.5 (0.08) | 1.92 (0.05)| 3.17 (0.06)| 10.64 (0.11)       |
| 35       | 88  | 6.10 (0.03)   |     | 45| 2.69 (0.14)| 2.0 (0.17) | 2.5 (0.50) | nd          | nd          | nd                  |

*Means followed by the same letter do not differ significantly from pairwise Wilcoxon rank test with Bonferroni-like correction (P > 0.01).
Table 2. Survival (S) in percentage at each stage of the nymph development, sex ratio in percentage (with binomial standard deviation), of *Nesidiocoris volucer*

| Temp (°C) | Overall survival | Sex ratio (N%) | 1st instar | 2nd instar | 3rd instar | 4th instar | 5th instar |
|-----------|------------------|---------------|-----------|-----------|-----------|-----------|-----------|
| 15        | 98.6 (92.3–99.9) | N             | 94.3a (86.0–98.4) | 94.3a (86.0–98.4) | 94.3a (86.0–98.4) | 94.3a (86.0–98.4) | 94.3a (86.0–98.4) |
| 25        | 96.0 (94.8–100.0) | 69             | 69             | 69             | 69             | 69             | 69             |
| 30        | 95.0 (94.8–99.9)  | 72             | 72             | 72             | 72             | 72             | 72             |
|           |                   | N             | 100 (94.8–100.0) | 100 (94.8–100.0) | 100 (94.8–100.0) | 100 (94.8–100.0) | 100 (94.8–100.0) |

 Bryce, Histed, and Aescht (2015) described the nymph development of *N. volucer* and reported that the nymphs hatched from eggs in 5–7 days. The nymphs were individually placed in plastic cups containing one leaf disc of the host plant but no prey (cotton, tomato, sweet pepper or tobacco, according to the prey rearing material). After 12 h for males and 24 h for female, each *N. volucer* adult was transferred in a similar plastic cup with *ad libitum* prey, and each adult was observed individually for 20 min with a dissecting microscope. The number of prey killed and predation behaviours (probing a prey and feeding on a prey) were recorded (Supplementary fig. S1: *N. volucer* feeding on *T. vaporariorum* nymphs). The following behaviours were also recorded and gathered in different classes: (i) the class ‘other feedings’ was gathering several behaviours: probing of the agar, probing of the leaf disc, probing of water droplets or probing on honeydew (produced by whiteflies); (ii) the class ‘contact’ was represented by behaviours of a contact made by the predator without feeding on the leaf disc; (iii) the class ‘other behaviours’ was represented by behaviours of resting, grooming and moving without probing; (iv) the last behaviour class was defined as ‘prey feeding’ including attack and consumption of the prey. The probing behaviour was referred here as the action of tasting different media by the predator. Each combination of prey species and *N. volucer* sex was represented by 14–20 replicate cups. The number of killed prey by *N. volucer* adults was analysed with a GLMM with a Poisson distribution, with the type of prey, the sex of the predator and their interaction as fixed effects and the experiment as a random effect. Because the interaction between sex and type of prey was significant, the effect of sex was tested for each type of prey and vice versa by a pairwise Tukey test with a Bonferroni-like correction.

**Effect of prey on development and longevity**

We determined the effect of prey species on the duration of *N. volucer* nymph development and the longevity of adults. The prey included larvae and adult thrips (*T. parvispinus*), adult spider mites (*Tetranychus sp.*), nymphs of two whitefly species (*B. tabaci* and *T. vaporariorum*) and *E. kuehniella* eggs. This experiment used newly *N. volucer* hatched nymphs and newly emerged adults that were placed individually in plastic cups (as described above) with the tested prey on a leaf disc of the host plant of the prey (see above insects section). The cups were kept at 25°C (in a climate chamber Sanyo, MLR-350), and tested prey and leaf discs were replaced twice each week. Leaf discs without prey were used as a control in each of the combination tested. The mortality of nymphs and adults was recorded daily. Each prey species was represented by 24–128 replicate cups for nymphs and by 25–88 replicate cups for adults (see Table 3). The longevity of *N. volucer* adults according to each prey was each analysed with a GLMM with a Poisson distribution, with the type of prey, the sex of the predator and their interaction as fixed effects and the experiment as a random effect. Because the interaction between sex and type of prey was significant, the effect of sex was tested for each type of prey and vice versa by a pairwise Tukey tests with a Bonferroni-like correction. The survival of *N. volucer* nymphs was tested with Bonferroni-like correction was used to compare weekly fecundity.

**Predation**

Predation by *N. volucer* adults was assessed on adult thrips (*T. parvispinus*), adult spider mites (*Tetranychus sp.*) and nymphs of two whitefly species (*B. tabaci* and *T. vaporariorum*), see insects section to have more details on each of the mentioned prey used in this section. Newly emerged adults of *N. volucer* were placed individually in plastic cups containing one leaf disc of the host plant but no prey (cotton, tomato, sweet pepper or tobacco, according to the prey rearing material). After 12 h for males and 24 h for female, each *N. volucer* adult was transferred in a similar plastic cup with *ad libitum* prey, and each adult was observed individually for 20 min with a dissecting microscope. The number of prey killed and predation behaviours (probing a prey and feeding on a prey) were recorded (Supplementary fig. S1: *N. volucer* feeding on *T. vaporariorum* nymphs). The following behaviours were also recorded and gathered in different classes: (i) the class ‘other feedings’ was gathering several behaviours: probing of the agar, probing of the leaf disc, probing of water droplets or probing on honeydew (produced by whiteflies); (ii) the class ‘contact’ was represented by behaviours of a contact made by the predator without feeding on the leaf disc; (iii) the class ‘other behaviours’ was represented by behaviours of resting, grooming and moving without probing; (iv) the last behaviour class was defined as ‘prey feeding’ including attack and consumption of the prey. The probing behaviour was referred here as the action of tasting different media by the predator. Each combination of prey species and *N. volucer* sex was represented by 14–20 replicate cups. The number of killed prey by *N. volucer* adults was analysed with a GLMM with a Poisson distribution, with the type of prey, the sex of the predator and their interaction as fixed effects and the experiment as a random effect. Because the interaction between sex and type of prey was significant, the effect of sex was tested for each type of prey and vice versa by a pairwise Tukey test with a Bonferroni-like correction.
was analysed with a pairwise $\chi^2$ test with a Bonferroni-like correction. A pairwise Wilcoxon rank test was done with a Bonferroni-like correction to compare nymph developmental time, according to sex and the type of prey.

### Injury of plants by *N. volucer* feeding

Feeding injury to plants caused by *N. volucer* was assessed under controlled conditions (i.e., constant number of insect per plant). The experiment was made on potted tomato plants in a greenhouse. Twelve newly emerged *N. volucer* females and 12 *N. volucer* males were collected (already mating) in the *N. volucer* rearing greenhouse and transferred in couple – in plastic cups. After 48 h, time considered as enough for the end of mating, the males and females were placed individually in a large insect-proof cap that enclosed the apical part of a 20 cm-tall tomato plant (one cap with one insect per plant). Twelve other tomato plants with no insect in the cap were used as blank controls. After 11 days (average time where adults of *N. volucer* died without prey nor leaf, results obtained in a preliminary test in lab conditions at 25°C), the caps and still alive *N. volucer* individuals were removed, and the plants were examined for damage. Damage looked for were as follows: number of brown rings and scars on the stem, petioles of leaves and leaflets. Each plant was grown for an additional 4 weeks during which it was examined for damage twice each week as described in Arnó *et al.* (2010) and compared to the growth development of the control plants.

### Results

#### Development of eggs and nymphs

The mean (SEM) developmental time of *N. volucer* eggs ranged from 27.59 (0.06) days at 15°C to 6.10 (0.03) days at 35°C (table 1). Mean egg developmental time significantly differed between all temperatures tested (all $P < 0.0001$), except between 30 and 35°C ($P = 0.75$). At 35°C, no nymphs survived beyond the 4th instar, and data from rearing at 35°C were only used to compare the survival of instars. The mean (SEM) duration of the entire developmental period for nymphs ranged from 49.41 (0.25) days at 15°C to 10.50 (0.07) days at 30°C (table 1). Pairwise comparisons of nymph development indicated significant differences between all temperatures, whatever the sex (all $P < 0.0001$, table 1). There was no difference between male and female at 25°C ($P = 0.85$), but a very slightly significant difference at 30°C ($P = 0.049$) was observed. A significant difference at 15 and 20°C ($P = 0.015$ and $P < 0.001$, respectively) was also observed.

The sex ratio was not significantly affected by temperature ($P = 0.976$). Overall survival of nymphs ranged from 94 to 100% (table 2) and did not depend on the temperature. Survival of instars was not significantly affected by temperatures between 15 and 30°C (all $P > 0.106$) but was significantly reduced ($P < 0.0001$) at 35°C (table 2).

#### Female longevity and fecundity

The average (SEM) female longevity was 27.12 (1.58) days, and the maximum longevity was 49 days. Fecundity in terms of numbers of nymphs that hatched ranged from 8 to 142 per female. The mean (SEM) number of total offspring produced per female was 65.0 (5.89) with a significant effect on the week ($P < 0.0001$, fig. 1). The first 3 weeks accounted for 81.9% of the total number of hatched nymphs, and these 3 weeks were each significantly different from the other weeks ($P < 0.001$ with weeks 4–6). Week 4 was significantly different from weeks 5 and 6 ($P < 0.001$). There was only one female laying eggs on week 7.

#### Predation and the effect of prey species and stage on *N. volucer* development and longevity

When the prey was *B. tabaci*, *Tetranychus* sp., *T. parvispinus* or *T. vaporariorum*, the average time spent exhibiting predatory behaviour (contact and prey feeding, fig. 2) was 64.1, 80.4, 41.8 and 85.6%, respectively, for *N. volucer* females and 54.5, 56.1,

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**Table 3.** Mean (SEM) nymph development time (in days), survival of nymphs and longevity of adults (in days) of *Nesidiocoris volucer* feeding on several prey stages

| Prey species | Stage of prey | N | Survival (95% CI) | Development time | Female | N | Longevity | Male | N | Longevity | P |
|--------------|--------------|---|------------------|------------------|--------|---|------------|------|---|------------|---|
| *B. tabaci*  | Nymph        | 49 | 61.2b (46.2–74.8) | 12.70 (0.18)c    | 25     | 16.32 (1.34)a | 24   | 11.38 (0.73)ab | 8.6) nd 55 | 8.89 (0.38)c | 56 | 7.75 (0.40)c | 0.591 |
| *E. kuehniella* | Egg         | 105 | 87.6c (79.8–93.2) | 11.19 (0.07)a    | 41   | 15.73 (1.19)a | 48   | 9.52 (0.52)bc | 10.3) nd 25 | 5.72 (0.45)e | 25 | 4.76 (0.33)d | <1 × 10⁻⁴ |
| *Tetranychus* sp. | Adult       | 41 | 0.0a (0.0–8.6) | nd | 55 | 8.89 (0.38)c | 56 | 7.75 (0.40)c | 11.53 (0.10)b | 76 | 12.46 (0.77)b | 72 | 12.10 (0.75)a | 0.989 |
| *T. parvispinus* | Larva       | 24 | 66.7b (44.7–84.4) | 14.94 (0.35)d | – | – | – | – | – | – | – | – | – |
| *T. parvispinus* | Adult       | 34 | 0.0a (0.0–10.3) | nd | 25 | 5.72 (0.45)e | 25 | 4.76 (0.33)d | 128 | 0.0a (0.0–2.8) | 98 | 6.52 (0.31)d | 91 | 5.60 (0.27)d | 0.068 |
| *T. vaporariorum* | Nymph       | 107 | 77.6bc (68.5–85.1) | 11.53 (0.10)b | 76 | 12.46 (0.77)b | 72 | 12.10 (0.75)a | 0.989 |

N, number of individuals; nd, no development; –, no data.

For nymph survival, survival within a column followed by the same letter does not differ significantly from a pairwise $\chi^2$ test with a Bonferroni-like correction ($P = 0.05$).

For nymph development time, means within a column followed by the same letter do not differ significantly from a pairwise Wilcoxon rank test with a Bonferroni-like correction ($P < 0.0001$).

For adult longevity, means within a column followed by the same letter do not differ significantly from a pairwise Tukey test ($P = 0.05$). The last column $P$ shows $P$-value of the effect of the predator sex on his longevity for each prey from pairwise Tukey test.
Species tested (fed with *B. tabaci* (*T. parvispinus* were not fed with prey or that were fed with *E. kuehniella* females lived significantly longer when fed *Nesidiocoris volucer* (table 3). Development times corresponded with the lowest survival rates (*N. volucer* eggs, *T. vaporariorum* nymphs, *B. tabaci* nymphs and *T. parvispinus* larvae (all \( P < 0.0001 \)). For *N. volucer* nymphs, the longest development times corresponded with the lowest survival rates (table 3).

The longevity of *N. volucer* adults was significantly affected by the interaction between sex and type of prey \(( P = 0.014 \) on the number of prey killed was observed. For each prey, the mean number killed did not significantly differ between *N. volucer* males and females (all \( P > 0.221 \)), except that significantly more *Tetranychus* sp. were killed by females than males \(( P < 0.0001 \) (fig. 2). Significantly fewer *T. parvispinus* than other prey were killed by females \(( P < 0.007 \)) and males \(( P < 0.007 \). Probing of the leaf disc and agar was observed regardless of prey type.

*Nesidiocoris volucer* nymphs died before reaching the adult stage when fed on adults of *Tetranychus* sp. or adults of *T. parvispinus*, and obviously with no prey (table 3). For the four other feedings, nymph survival was 61.2, 66.7, 77.6 and 87.6% for nymph fed with *B. tabaci*, instars of *T. parvispinus*, *T. vaporariorum* or *E. kuehniella*, respectively. Mean developmental time did not significantly differ between male and female nymphs whatever the feeding (all \( P > 0.254 \)). Mean development duration significantly differs for *N. volucer* nymphs fed with *E. kuehniella* eggs, *T. vaporariorum* nymphs, *B. tabaci* nymphs and *T. parvispinus* larvae (all \( P < 0.0001 \)). For *N. volucer* nymphs, the longest development times corresponded with the lowest survival rates (table 3).

The longevity of *N. volucer* adults was significantly affected by the interaction between sex and type of prey (\( P < 0.0001 \)). *Nesidiocoris volucer* females lived significantly longer when fed with *E. kuehniella*, or *B. tabaci*, than when fed with the other species tested (\( P < 0.0001 \), and males fed with *T. vaporariorum* \(( P < 0.0001 \). Longevity was shorter for males and females that were not fed with prey or that were fed with *T. parvispinus* \(( P < 0.0001 \). Females lived significantly longer than males when fed with *B. tabaci* \(( P < 0.001 \) or with *E. kuehniella* \(( P < 0.0001 \) (table 3).

### Discussion

The results of this study suggest that the native mirid *N. volucer* may be an effective predator of insect pests in greenhouses in La Réunion and might probably be in other tropical areas such as it was observed in the field in Niger (Garba *et al.*, 2020). The establishment and performance of biological control agents are greatly affected by temperature (Bale *et al.*, 2009; Hughes *et al.*, 2009), and we found that temperature significantly influenced the developmental time of the immature stages of *N. volucer*. Development from egg to adult required only 17 days at 30°C but 77 days at 15°C. However, neither sex ratio nor nymph survival was influenced by temperature (except nymphs failed to develop at 35°C); nymph mortality was \(<6\% at temperatures between 15 and 30°C. Reunion is known for its multitude of climates and its significant temperature changes, which are greatly affected by altitude (Robert, 2003). Our results suggest that this indigenous insect might be well adapted to the multiple environmental conditions found on the island. On the other hand, the rate of development was much greater at 30°C than at 15°C (the lower temperature tested), suggesting that *N. volucer* populations might be more effective as biological control agents in the warmer, low altitude parts of the island.

The effects of temperature on the duration of *N. volucer* immature stages reported here are similar to those previously reported for *N. tenuis* (Hughes *et al.*, 2009; Sanchez, 2009). The latter authors, however, reported much higher mortality for immature stages of *N. tenuis* than we recorded for *N. volucer*, i.e. *N. tenuis* mortality was 48% at 15.5°C and 37% at 15°C. The thermophilic nature of *N. tenuis* means that it is unlikely to establish in cooler areas (Hughes *et al.*, 2009, 2010; Sanchez, 2009), and about 50% of its nymphs were able to mature to the adult stage at 35°C (Sanchez, 2009), which was not the case for *N. volucer* in the...
current study. Nevertheless, the high survival of *N. volucer* immature stages on a large range of temperature could make it as effective as or more effective than *N. tenuis* in greenhouses in tropical area.

To be effective, a biological control agent should have a high reproductive potential (Hastings, 1997; Uneke, 2007). *Nesidiocoris volucer* females produced an average of 65 viable eggs (as indicated by the numbers of nymphs that hatched) at 25°C, and the maximum recorded in our study was 142. More eggs (as indicated by the numbers of nymphs that hatched) at 25°C, with a mean (SEM) of 60.0 (5.00) eggs per female produced 79.5 (5.50) and 68.0 (4.99) eggs per female, respectively (Sanchez et al., 2009). Additional research is needed to determine how *N. volucer* fecundity is affected by temperatures other than 25°C and especially at lower temperatures. Given that *N. tenuis* is successfully mass reared and given the *N. volucer* fecundity recorded here, we suspect that fecundity will not be a limiting factor for the mass rearing of *N. volucer*.

In the current study, we investigated *N. volucer* predation on a local range of prey, among the main insect pest of greenhouse-produced tomatoes in La Réunion (Delatte et al., 2007). Although our observations indicate that *N. volucer* males and females fed on each of the four prey, the prey type and stage significantly influenced the duration of *N. volucer* nymph instars and the longevity of *N. volucer* adults. However, nymph development was fastest when *N. volucer* females fed on *E. kuehniella* eggs. This food has often been used to feed predacious insects because of its high protein content (Morales-Ramos et al., 2014). However, *E. kuehniella* eggs are expensive and cannot be used for the mass rearing of predators. Still, *E. kuehniella* eggs could be useful to help establish an *N. volucer* population in a greenhouse or as a complementary food (Urbanea-Bernat et al., 2015).

Among all pests tested as prey for *N. volucer*, the whiteflies *B. tabaci* and *T. vaporariorum* supported the fastest nymph development, one of the highest survival rate, and the greatest adult longevity. Similar results were observed for *N. tenuis* (Perdiks and Arvanit, 2016). Although numbers of *Tetranychus* sp., *B. tabaci* and *T. vaporariorum* killed by *N. volucer* adult predators were not significantly different in the current study, *N. volucer* nymphs could not complete their development and adults had shorter lifespans when fed with *Tetranychus* sp. This suggests that *Tetranychus* sp. might not provide sufficient nutrients for the development and maximal survival of *N. volucer*. Other studies have demonstrated that the type of prey can greatly affect predator development and reproduction (Bonte et al., 2015; Ugine et al., 2018). In the current study, *N. volucer* nymphs developed on *T. parvispinus* larvae but not on *T. parvispinus* adults, which are more mobile than the larvae. Although *N. volucer* was able to predate *Tetranychus* sp. and adults of *T. parvispinus*, our results suggest that the complete development of nymphs on these prey would require complementary food like *E. kuehniella*.

Several studies have found that biological control tended to be stronger when agents were generalists rather than specialists (Symondson et al., 2002; Stiling and Cornelissen, 2005). *Nesidiocoris tenuis* is mostly used against whiteflies on tomato, but can be considered as a generalist predator, i.e. it can contribute to the control of thrips, leafminers, spider mites and other lepidopterans in the greenhouse (Rüdavets and Castañé, 1998; Schaefer and Panizzi, 2000; Calvo et al., 2009; Hassanpour et al., 2016). This generalist predator is closely related to *N. volucer*, and (i) regarding the current results, (ii) the recent field study conducted in Niger that has detected *N. volucer* in tomato fields infested with *Tuta absoluta* (Lepidoptera: Gelechiidae) (Garba et al., 2020) and (iii) previous studies on *N. tenuis*, it tends to indicate that the prey range is similar for the two species, placing *N. volucer* as a potential efficient generalist predator.

A biological control agent should control the pest without detrimental side effects (Gurr and Wratten, 2012; Van Lenteren et al., 2018). Many mirids are zoophytophagous and can cause economically significant damage to crops (Wheeler, 2001; Castañé et al., 2011). *Nesidiocoris tenuis*, for example, causes necrotic rings, flower abortion, reduced growth and small fruits (Sanchez and Lacasa, 2008; Calvo et al., 2009; Arnó et al., 2010). Some predators
use plant material to complement or supplement their diet in order to enhance their fitness (Maleki et al., 2006; Ugine et al., 2018). In our study, N. volucer nymphs could not complete their development without prey, and adult longevity was quite reduced in the absence of prey even when plant tissue was available. These results demonstrate that N. volucer requires animal prey as a component of its diet. We observed that N. volucer punctured the leaf discs even when prey were present. Perhaps N. volucer obtains supplemental food or water from plants. We suspect that water may be important because predaceous heteroptera use extra-oral digestion and therefore require a substantial amount of water to feed on their prey (Cohen, 1995). They also need water to maintain their physiological status. This required water is mainly obtained from plant tissues (Castañé et al., 2011). When N. volucer adults were contained on the top of tomato plants without prey, they did not cause visible damage to the plants even after 6 weeks. However, this experiment did not allow us to test for all possible damage on tomato plants (i.e. flower abortion). Mirids could injure different parts of the plant (stems, leaves, fruits, flowers), and the injury might differ among mirid stages. Additional observations of a N. volucer population in a tomato greenhouse during the entire 6-month growing cycle were made (authors’ personal communication) and indicated that N. volucer did not damage any plant, even though the numbers of N. volucer nymphs sometimes exceeded 100 per plant. Arnó et al. (2006) observed that the zoophytophagous N. tenuis caused nectric rings on tomato, and that the damage was positively correlated with the number of adult mirids. Although N. tenuis and N. volucer are closely related, our observations suggest that they differ in their direct effects on plants. If N. volucer is able to regulate pest numbers without damaging host plants, it could be a very useful biological control agent. Additional research is still needed on how N. volucer affects pest numbers and crop yields.

This study has shown that N. volucer has thermal plasticity, can reproduce and develop in tomato greenhouses, does not damage the plants supporting its prey, and is a generalist predator that feeds on T. vaporariorum, B. tabaci, T. parvispinus and Tetranychus sp. The results suggest that N. volucer could be useful for the biological control of insect pests in tomato greenhouses of La Réunion and a candidate could be tested in other tropical environments where it is also present (Schuh, 2002–2013). The effectiveness of N. volucer could be increased by applying it with other biological control agents. Based on a meta-analysis, Stiling and Cornelissen (2005) found that the addition of two or more biological control agents would increase mortality by 12.97% and decrease pest abundance by 27.17% compared to the addition of one biological control agent. Only two parasitoids, E. formosa and E. eremicus, are currently mass reared to control whiteflies on La Réunion; biological control of whiteflies might be increased by complementing these parasitoids with N. volucer. Rearing of this mirid, in small quantity, is already successful on tobacco and it has been efficiently established in a tomato greenhouse for 6 months, revealing our ability to raise it. As a whole, all those results have shown to present this mirid as a new generalist predator for tomato pests under greenhouse (with great suitability for tropical and subtropical climates), its development in mass-rearing facilities has started in La Réunion.

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