



Comparison of prey consumption by *Dicyphus tamaninii* reared conventionally, and on a meat-based diet

Cristina CASTAÑÉ, Javier IRIARTE and Eric LUCAS*

Departament de Protecció Vegetal, IRTA-Centre de Cabrils, Ctra. de Cabrils s/n, 08348
Cabrils (Barcelona), Spain

*Author for correspondence; Département des Sciences Biologiques, Université du Québec à
Montréal, CP 8888, Succ. "Centre-Ville" Montréal, Québec, Canada H3C 3P8

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Abstract. The zoophytophagous predator *Dicyphus tamaninii* Wagner (Heteroptera: Miridae) has been successfully reared for more than five generations on a meat-based diet, and in the absence of a plant as a substrate for oviposition and feeding. We compared the predation efficiency of *D. tamaninii* produced on this diet with those reared conventionally on *Ephestia kuehniella* eggs (Zeller) (Lepidoptera: Pyralidae) and tobacco plants. Their performances were evaluated on two prey, the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) and the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae). Their tendency to cannibalistic behaviour was also evaluated. Nymphs of *D. tamaninii* produced on the meat diet consumed a similar number of greenhouse whitefly pupae after 24 and 48 hours to nymphs reared by the conventional method. Diet-reared females consumed significantly more whitefly pupae after 24 and 48 hours than control females. When the cotton aphid was offered, diet-reared *D. tamaninii* nymphs and females consumed similar numbers of prey to control *D. tamaninii*. There was no significant increase in cannibalistic behaviour of diet-reared *D. tamaninii* after 3 and 5 days of interaction. These results show that, after five generations, the predation efficiency and the tendency to cannibalism of meat-reared *D. tamaninii* is similar to that of conventionally-reared individuals.

Key words: artificial diet, cannibalism, *Dicyphus tamaninii*, predation capacity, zoophytophagous predator

Introduction

Dicyphus tamaninii Wagner (Heteroptera: Miridae), a polyphagous predator present in the Mediterranean area, is an effective biological control agent of vegetable crop pests (Alomar and Albajes, 1996; Castañé et al., 2000a, b). This zoophytophagous insect oviposits inside the pedicel or vein tissues of the plant, preys on several arthropod pest species and also feeds on the plant itself (Albajes et al., 1996; Montserrat et al., 2000a; Lucas and Alomar,

2001). It is commonly reared on tobacco, with *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs as prey (Agustí, 1998), as is another closely-related species, *Macrolophus caliginosus* Wagner, a widely-used predator in vegetable crops in European horticulture. The requirement for plants for feeding and reproduction, together with the use of expensive prey (such as eggs of *E. kuehniella*), raises the cost of production and limits their use as biological control agents.

A meat-based diet has been successfully tested for rearing *D. tamaninii*, for more than five continuous generations (Iriarte and Castañé, 2001). This diet has already been described for rearing *Podisus maculiventris* (Say) (De Clercq et al., 1998) and is a modification of Cohen's (1985) diet for rearing *Geocoris punctipes* (Say). Dental cotton rolls, as described by Constant et al. (1996), were offered as oviposition substrate and no plant material was used in the entire process. Reproduction was good and colonies grew well. It is now important to evaluate the quality of the insects produced as biological control agents.

In the present study we evaluated the prey consumption of *D. tamaninii* obtained from the 5th generation of a continuous artificial rearing on a meat diet, in comparison with *D. tamaninii* reared using the standard method on tobacco plus *E. kuehniella* eggs. The prey offered were two common pests of horticultural crops: the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) and the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae). Cannibalistic behaviour was also evaluated since low food quality has been identified as a significant factor in the occurrence of cannibalism (Fox, 1975).

Materials and methods

Rearing D. tamaninii and its prey

Diet-reared *D. tamaninii* were collected from the 5th generation of a continuous culture on a meat diet composed of fatty ground beef, beef liver, sucrose, ascorbic acid and hen's egg yolk presented in stretched Parafilm® (De Clercq et al., 1998). Water was supplied in a moistened cotton wool, and dental cotton rolls wrapped in Parafilm® served as oviposition substrates (Constant et al., 1996). Control *D. tamaninii* were collected from a culture maintained in the standard way on tobacco plants, with *E. kuehniella* eggs as prey. We maintained diet- and control-predator colonies and conducted all experiments under controlled conditions of 25 ± 1 °C, and $70 \pm 10\%$ RH, and a photoperiod of 16:8 (L:D). *T. vaporariorum* was reared on tobacco plants, and *A. gossypii* on cucumber plants, in a heated greenhouse.

Predation on T. vaporariorum and on A. gossypii

To evaluate the predation efficiency of diet-reared *D. tamaninii* in relation to control *D. tamaninii*, two experiments were performed in which *T. vaporariorum* and *A. gossypii* were offered as prey. Arenas consisted of a cucumber leaf disc (6.5-cm-diameter) placed upside down on a 2 cm thick layer of agar (0.5%) in a 7-cm-diameter cage with ventilation. The number of prey consumed was recorded after 1, 4, 24, and 48 hours.

In a first experiment, 20 whitefly pupae were offered to one 4th instar nymph of *D. tamaninii* every 24 hours and the test was repeated 12 times. Twenty five whitefly pupae were offered to one 7–14 day old adult female of *D. tamaninii* every 24 hours. The test was repeated 9 times. The number of whitefly pupae offered was calculated according to consumption rates observed in Montserrat et al. (2000b) and it was in excess of requirements.

In a second experiment 15 apterous and 15 alate *A. gossypii* were offered to one newly moulted 4th instar nymph of *D. tamaninii* every 24 hours and the test was repeated 10 times. Forty apterous aphids were offered to one 7–14 day old adult female of *D. tamaninii* every 24 hours and the test was repeated 10 times. The number of aphid nymphs offered was calculated according to consumption rates observed in Alvarado et al. (1997) and it was in excess of requirements.

The number of moulted nymphs was recorded in both experiments and a Student's *t*-test was applied to compare the prey consumption between control- and diet-reared *D. tamaninii*.

Cannibalism

In order to determine the cannibalistic behaviour of predators reared on both diets, three 1st instar nymphs of the predator, taken from the conventional rearing, were offered to one 4th instar nymph or to one adult female of *D. tamaninii*. A control with only three 1st instar nymphs of the predator was included. No prey were offered in any of these treatments. Arenas were similar to those previously described. The test was repeated 10 times and the number of dead 1st instar nymphs was recorded daily during 7 days. The proportion of cannibalised nymphs was compared using a likelihood ratio G-test at 1, 3 and 5 days of interaction, since too few nymphs remained alive after this time.

Results

Predation on T. vaporariorum

Control and diet-reared fourth instar nymphs and females of *D. tamaninii* actively consumed whitefly pupae (Figure 1 a and b). Control *D. tamaninii* nymphs consumed significantly more pupae than diet-reared *D. tamaninii* nymphs after the first four hours of the test ($t = -2.34$; $df = 18.9$ $p = 0.030$) while consumption was similar at 1 ($t = -1.77$; $df = 22$; $p = 0.090$), 24 ($t = 0.69$; $df = 19.6$; $p = 0.498$) and 48 hours ($t = 1.69$; $df = 15.9$; $p = 0.110$). There was considerable variability in predation among individuals since some nymphs moulted during the experiment (9% of control *D. tamaninii* and 33.3% of diet-reared *D. tamaninii* nymphs) and during this time they stop feeding. In the case of females, diet-reared *D. tamaninii* consumed significantly more whitefly pupae than control *D. tamaninii* after 24 hours (30.1 ± 2.25 vs 20.4 ± 1.83 whitefly pupae, respectively) ($t = 3.37$; $df = 15$; $p = 0.004$), and this difference increased after 48 h (58.1 ± 3.67 vs 39.2 ± 0.92 whitefly pupae, respectively) ($t = 4.18$; $df = 15$; $p = 0.001$). Their consumption was similar at 1 ($t = 0.09$; $df = 15$; $p = 0.933$) and 4 hours ($t = 1.55$; $df = 15$; $p = 0.142$).

Predation on A. gossypii

Control and diet-reared fourth instar nymphs and females of *D. tamaninii* also actively consumed *A. gossypii* (Figure 2, a and b). There were no significant differences between diet-reared *D. tamaninii* and control *D. tamaninii* nymphs in consumption of total *A. gossypii* after 1 ($t = -0.67$; $df = 18$; $p = 0.511$), 4 ($t = -0.94$; $df = 18$; $p = 0.361$), 24 ($t = 0.54$; $df = 18$; $p = 0.593$) and 48 hours ($t = -0.77$; $df = 18$; $p = 0.453$).

Predation by nymphs on apterous *A. gossypii* was significantly higher than on the alate form after 1 hour for control *D. tamaninii* ($t = -3.29$; $df = 18$; $p = 0.004$), and after 48 hours for diet-reared *D. tamaninii* ($t = -2.29$; $df = 18$; $p = 0.034$). Both diet-reared and control nymphs had consumed approximately twice as many total apterous aphids as alate aphids by the end of the assay. Diet-reared and control *D. tamaninii* adult females were not significantly different in their consumption of apterous *A. gossypii* after 1 ($t = 0.32$; $df = 17$; $p = 0.753$), 4 ($t = -0.54$; $df = 16$; $p = 0.598$), 24 ($t = -0.44$; $df = 16$; $p = 0.667$) and 48 hours ($t = -1.08$; $df = 16$; $p = 0.296$).

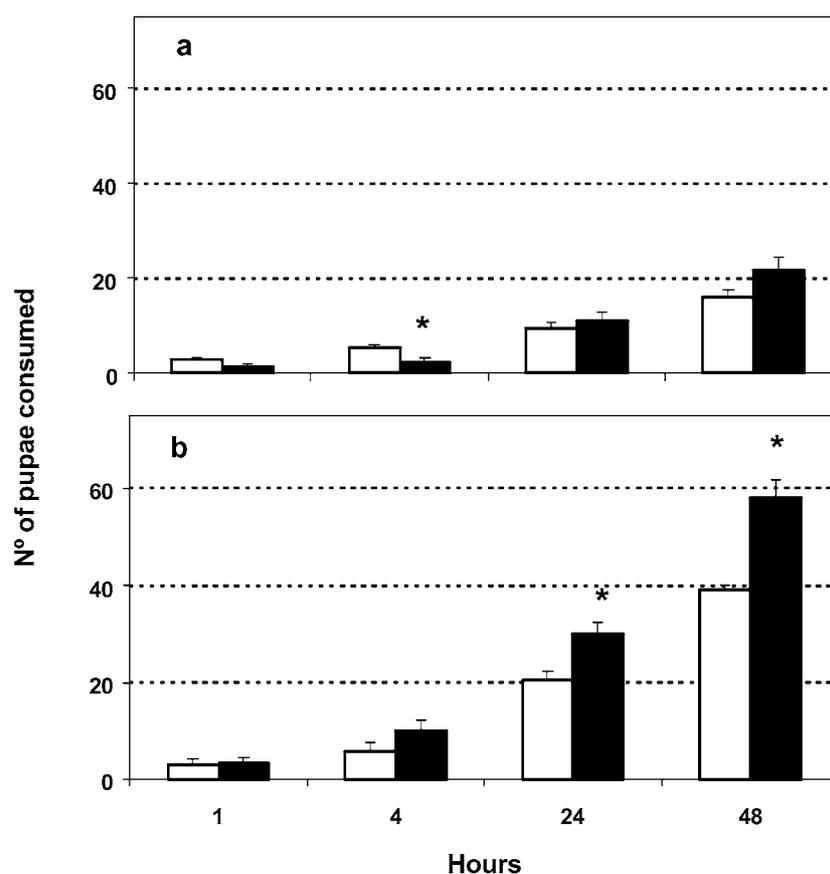


Figure 1. Cumulative number of greenhouse whitefly pupae (*Trialeurodes vaporariorum* Westwood) consumed after 1, 4, 24 and 48 hours by 4th instar nymphs (a) ($n = 12$) or adult females (b) ($n = 9$) of *Dicyphus tamaninii* Wagner. White bars represent individuals reared on a conventional diet and black bars individuals reared on a meat diet. * statistical differences ($p < 0.05$).

Cannibalism

The proportion of *D. tamaninii* 1st instar nymphs consumed either by 4th instar nymphs (Figure 3a), or by adult females (Figure 3b) was similar in the diet-reared group and in the control group. The only difference occurred after 1 day where the diet-reared *D. tamaninii* 4th instar nymphs showed a significantly higher tendency to prey on the first instar than did 4th instars of control *D. tamaninii* ($G = 5.94$; $df = 1$; $p = 0.015$).

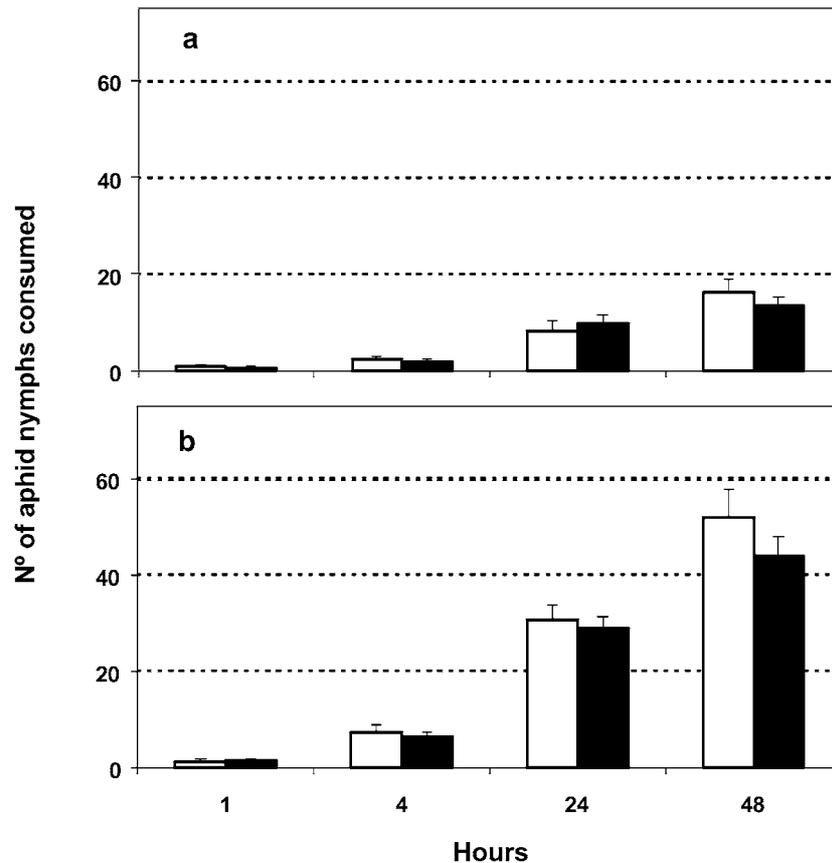


Figure 2. Cumulative number of *Aphis gossypii* Glover consumed after 1, 4, 24 and 48 hours by 4th instar nymphs (a) (apterous + alate) ($n = 10$) or adult females (b) (apterous) ($n = 10$) of *Dicyphus tamaninii* Wagner. White bars represent individuals reared on a conventional diet and black bars represent individuals reared on a meat diet.

Discussion

In comparison with predators reared on a standard diet on a tobacco plant with eggs of *E. kuehniella*, the continuous rearing of *D. tamaninii* on a meat-based diet did not decrease its prey consumption on greenhouse whitefly pupae and on *A. gossypii* (apterous or alate), two common prey of this predator in natural and agricultural systems (Albajes et al., 1996; Alvarado et al., 1997). Therefore, meat-reared *D. tamaninii* were as effective as predators of the pests tested as were conventionally-reared insects. Other predators reared on a meat-based diet were shown to be as effective in prey consumption as predators reared on conventional hosts, as was the case for *Geocoris*

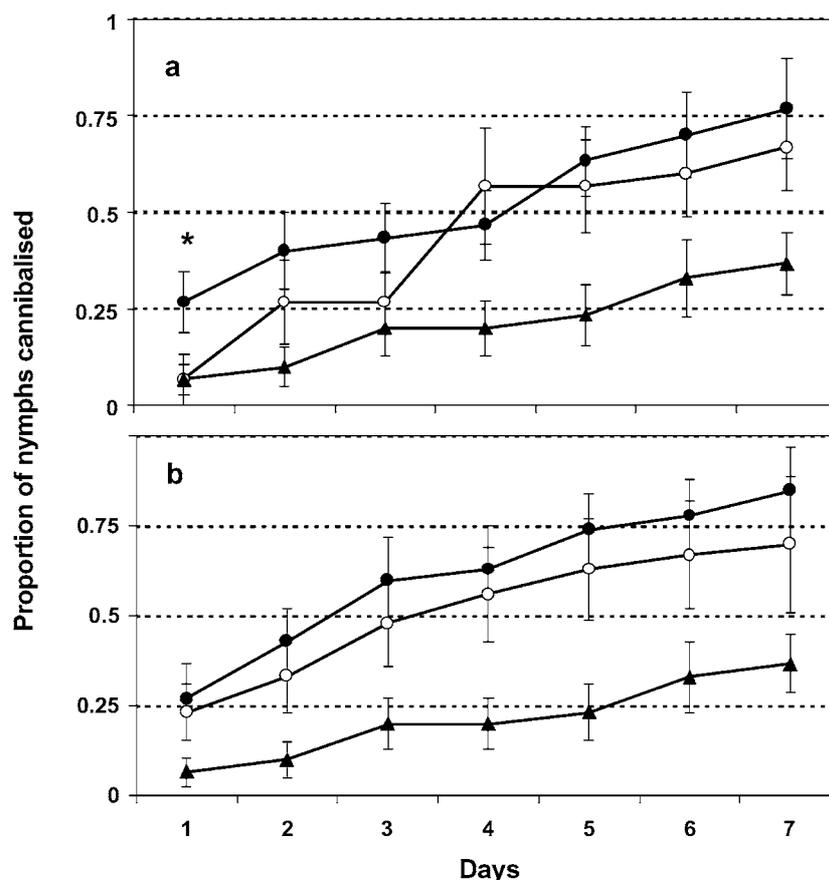


Figure 3. Cumulative proportion of cannibalised 1st instar nymphs of *Dicyphus tamaninii* Wagner by 4th instar nymphs (a) or adult females (b) of *D. tamaninii* during seven days. —○— individuals reared on a conventional diet, —●— individuals reared on a meat diet, —▲— cannibalism among 1st instar nymphs. ★ statistical differences ($p < 0.05$).

punctipes (Say) (Hagler and Cohen, 1991), *Chrysoperla rufilabris* Burmeister (Cohen and Smith, 1998), *Podisus maculiventris* (Say), *P. sagitta* (Fabricius) (De Clercq and Degheele, 1993) and *P. nigrispinus* (Dallas) (Saavedra et al., 1997).

We found a significantly higher predation rate on one species of prey (whitefly pupae) by meat-reared *D. tamaninii* in comparison with conventionally-reared predators, and similar results have also been documented by other authors with this type of diet. Thus, *P. nigrispinus* nymphs reared on an artificial diet consumed significantly more *Anticarsia gemmatalis* Hübner (Lepidoptera Noctuidae) larvae (Saavedra et al., 1997) and

P. maculiventris nymphs reared on an artificial diet consumed significantly more *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae (Chocorosqui and De Clercq, 1999) than did conventionally-reared insects. It is possible that some small imbalance in the nutrition of meat-fed predators may generate a higher predation rate on whitefly pupae. Alternatively, the *E. kuehniella* eggs diet (which is not the natural diet of *D. tamaninii*) could be inferior to the meat-based diet, resulting in weaker predators with reduced predation potential.

Food quality has been identified as a significant factor in the occurrence of cannibalism (Fox, 1975; Polis, 1981; Joyner and Gould, 1987). Among zoophytophagous species, cannibalism in the field may be rare, since predators can survive by feeding on plants, usually an abundant food (see Lucas and Alomar, 2001). In the case of *D. tamaninii*, previous studies have shown that their tendency to intraguild predation (a type of interaction very similar to cannibalism) was less marked than for strictly zoophagous species (Lucas and Alomar, unpublished). If cannibalism is a response to some deficiency in the artificial diet, it may disappear after a period of cannibalism has restored the necessary nutrients. This hypothesis gains support from our experiments where no difference was observed between the two groups after 24 hours of cannibalism.

There has been controversy about the quality of predators reared on artificial diets in relation to their efficiency in the field, but, as discussed by Cohen (2000), these predators have been shown to be as efficient as those reared on conventional prey. Furthermore, they can be similar to field individuals as was shown by Hagler and Cohen (1991) who found similar prey preferences for meat-reared *G. punctipes* compared with field-collected females. Continuous rearing on artificial media does not produce predators that prefer the diet to living prey. In fact, Cohen and Smith (1998), observed that *Chrysoperla rufilabris* Burmeister preferred live prey (*Bemisia argentifolii* Bellows and Perring, *A. gossypii* or *Trichoplusia ni* Hüb.) to a meat-based diet.

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