Institut für Pflanzenkrankheiten der
Rheinischen Friedrich-Wilhelms-Universität zu Bonn

Biological and ecological studies on the polyphagous predatory bug *Dicyphus tamaninii* WAGNER (Heteroptera: Miridae) as a natural enemy of the melon aphid *Aphis gossypii* GLOVER (Homoptera: Aphididae)

Inaugural–Dissertation

zur

Erlangung des Grades

Doktor der Agrarwissenschaften

(Dr. agr.)

der

Hohen Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität

zu Bonn

vorgelegt am 06. 05. 2002

von

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Tag der mündlichen Prüfung: 13. 06. 2002

Gedruckt bei:

Biological and ecological studies on the polyphagous predatory bug *Dicyphus tamaninii* WAGNER (Heteroptera: Miridae) as a natural enemy of the melon aphid *Aphis gossypii* GLOVER (Homoptera: Aphididae)

Abstract

The present research aimed to study the biology and prey consumption by *Dicyphus tamaninii* WAGNER (Heteroptera: Miridae) by feeding on different age groups of *Aphis gossypii* GLOVER (Homoptera: Aphididae) as prey at different temperatures in the laboratory. The host plant spectrum for oviposition by the predator was also investigated. Moreover, experiments were conducted to study the interaction of *D. tamaninii* with other natural enemies and the potential damage it might cause to cucumber. Finally greenhouse experiments were carried out in order to investigate the efficiency of the predator under more natural conditions.

The results of the laboratory experiments showed that *D. tamaninii* was able to successfully feed, develop and reproduce when exclusively fed on *A. gossypii* as prey at a wide range of low and high temperatures, however a constant temperature of 35±1°C was fatal to the predator. The predatory bug was able to adapt smoothly to fluctuating prey offer and maintain its capability of oviposition even when the prey was relatively scarce. The average daily prey consumption by the late nymphal instars as well as the adult stage of the predator on *A. gossypii* was high, where it reached up to 60 aphid/day by the adult female. Although *D. tamaninii* showed a clear affinity toward *A. gossypii*, it accepted also other important pest species offered as prey. Although cucumber, tobacco and tomato were preferred, the predatory bug seems to occupy a wide plant host range for oviposition. Despite being polyphagous, the predatory bug is not likely going to disrupt the efficiency of some other natural enemies in a biological control program. Investigating the plant-feeding habit of the predator showed that, *D. tamaninii* preferred leaves to cucumber fruits, a fact that leads to the conclusion that the potential damage to cucumber fruits by the phytophagous predator remains low.

In the greenhouse experiments, it was found that the release of 2 *D. tamaninii* adults per plant was sufficient to achieve up to 90% reduction in *A. gossypii* population, when the predator was released 1 week after infestation with the aphid. Nevertheless, it is speculated that an earlier release of *D. tamaninii* would be more effective in the biological control of *A. gossypii*.

In conclusion, *D. tamaninii* exhibited under laboratory as well as greenhouse conditions many features, which make the predatory bug a very promising natural enemy to be used in framework of a biological control program of the melon aphid on cucumber.

Biologische und ökologische Untersuchungen über die polyphage, räuberische Wanze *Dicyphus tamaninii* WAGNER (Heteroptera: Miridae) als Gegenspieler der Grünen Gurkenlaus *Aphis gossypii* GLOVER (Homoptera: Aphididae)

Kurzfassung

Das Ziel der vorliegenden Arbeit war es, die Biologie und Prädationsleistung von *Dicyphus tamaninii* WAGNER (Heteroptera: Miridae) während ihrer Entwicklung und Lebensdauer mit verschieden alten Individuen von *Aphis gossypii* GLOVER (Homoptera: Aphididae) als Beute bei unterschiedlichen Temperaturen im Labor zu untersuchen. Weiterhin wurden Untersuchungen über das Wirtspflanzenspektrum zur Oviposition, das Verhalten von *D. tamaninii* gegenüber anderen Nützlingen und die potentiellen Saugschäden an Gurkenfrüchten, die durch den Räuber ebenfalls angerichtet werden können, durchgeführt. Abschließend erfolgte eine Erfolgskontrolle der Raubwanze in Gewächshausversuchen.

Die Ergebnisse der Laboruntersuchungen zeigten, dass die Raubwanze in der Lage ist, sich erfolgreich mit *A. gossypii* als Beute bei allen getesteten Temperaturen zu ernähren, zu entwickeln und zu vermehren. Lediglich bei der Temperatur 35±1°C starben alle räuberischen Entwicklungsstadien. *D. tamaninii* besitzt eine beachtliche Fähigkeit zur Eiablage, auch wenn das Beuteangebot relativ niedrig ist. Die durchschnittliche tägliche Prädationsleistung von *D. tamaninii* war im allgemeinen hoch, wobei ein Weibchen bei einer Temperatur von 30±1°C bis zu 60 Aphiden/Tag vertilgte. In den Untersuchungen zur Beutepräferenz von *D. tamaninii* war *A. gossypii* deutlich bevorzugt. Obwohl Gurken-, Tabak- und Tomatenpflanzen deutlich bevorzugt wurden, scheint es, dass die Raubwanze ein weites Wirtspflanzenspektrum zur Oviposition besetzt. Weiterhin ist eine negative Auswirkung auf die Effizienz von einigen anderen Nützlingen unwahrscheinlich. Die durch *D. tamaninii* an Gurken verursachten Saugschäden waren unbedeutend. Außerdem bevorzugten die Raubwanzen die Gurkenblätter vor den Früchten.

In den Gewächshausversuchen konnte eine Reduzierung von bis zu 90% der Population von *A. gossypii* bei Freilassung von zwei adulten *D. tamaninii* pro Gurkenpflanze erreicht werden, wenn die Freilassung eine Woche nach der Infestierung mit den Aphiden erfolgte.

Zusammenfassend zeigte *D. tamaninii* sowohl im Labor als auch im Gewächshaus zahlreiche positive Eigenschaften, die sie als erfolgsversprechenden Räuber im Rahmen biologischer Bekämpfungsprogramme gegen *A. gossypii* kennzeichnen.

CONTENTS Page

1 INTRODUCTION

The melon aphid *Aphis gossypii* GLOVER (Homoptera: Aphididae) is a cosmopolitan polyphagous pest, generally distributed throughout the tropical, subtropical and temperate zones (YORK 1992, HAGVAR and HOFSVANG 1994). In the temperate zone it is principally a pest of vegetables and ornamental plants in both open field and greenhouse (VAN STEENIS 1992, LECLANT and DEGUINE 1994). In Germany, the aphid had been reported as a serious pest of cucumber under greenhouse in 1990 (DENGLER 1991, ALBERT and MERZ 1995, BÜNGER 1996).

Currently, *A. gossypii* is becoming more important pest because of increased insecticide tolerance and destruction of natural enemies through the use of pesticides in cotton and vegetable plantations (KING et al. 1987, SLOSSER et al. 1989). Chemical control of the melon aphid is therefore inconsistent and expensive (HARDEE 1993). Although factors other than destruction of natural enemies have been implicated in inducing melon aphid outbreaks (KERNS and GAYLOR 1993), arthropod predators and parasitoids have a key role in regulating its populations (WELLINGS and DIXON 1987). Therefore, current research is directed toward the development of management systems that use biological control methods.

More attention has been paid to insect parasitoids than to predators, a disparity seen in classic and recent research works (WAAGE and GREATHEAD 1986). Most research on insect predators has been devoted to lady beetles (Coccinellidae) and green lacewings (Chrysopidae), relatively few studies have focused on predatory Heteroptera (BARBOSA et al. 1994, COLL and RUBERSON 1998a). In recent years, however, there has been an increased interest in predatory bugs from both ecological and applied perspectives. A growing body of literature indicates that these predators are an important component of the arthropod fauna in both natural and managed habitats. Some attributes of the predatory bugs, such as plant feeding, promise to widen our views about biological control. The commercial availability and use of predatory bugs in augmentative biological control programs has increased from 2 species in 1989 to more than 10 in 1995 (COLL and RUBERSON 1998a)

Among many natural enemies, which are either being already in use or being tested for the biological control of *A. gossypii*, the polyphagous bug *Dicyphus tamaninii* WAGNER (Heteroptera: Miridae) was found to be a promising predator of this pest (ALVARADO et al. 1997, CASTANE et al. 1997, SALEH and SENGONCA 2000, 2001a, b, SENGONCA and SALEH 2002, SENGONCA et al. 2002). The polyphagous bug had been recognised as a predator of the greenhouse whitefly *Trialeurodes vaporariorum* WESTWOOD (Homoptera: Aleyrodidae) on tomato since early 1980s (GABARRA et al. 1988, ALOMAR et al. 1990, 1991, 1994). Its effectiveness as a common predator of the western flower thrips *Frankliniella occidentalis* (PERGANDE) (Thysanoptera: Thripidae) had also been documented (GABARRA et al. 1995, CASTANE et al. 1997). However, sufficient knowledge of this predator is still lacking in the literature.

The present work aimed, therefore, to study the biology and prey consumption by *D. tamaninii* during its development and longevity by feeding on different age groups of *A. gossypii* as prey at two different constant temperatures of 25±1°C and 18±1°C. Further experiments were devoted to study the effect of extreme constant and alternating temperatures on the development and prey consumption by *D. tamaninii*. The effect of the daily prey offer on fecundity as well as prey preference and alternative nutritional sources for the predator were also investigated. Moreover, experiments were conducted to investigate the interaction of *D. tamaninii* with other natural enemies and the potential damage it might cause to cucumber fruits under different nutritional conditions. Finally greenhouse trials were carried out in order to confirm the efficiency of *D. tamaninii* for the biological control of *A. gossypii* on protected cucumber. All these experiments have the goal of comprehensively studying the potential of this promising predator for the biological control of *A. gossypii*, and hopefully, to develop a new successful biological control possibility of this stubborn pest.

2 MATERIAL AND METHODS

2.1 Laboratory experiments

2.1.1 Rearing of the insects for experiments

2.1.1.1 Rearing of *Aphis gossypii*

2.1.1.1.1 Stock culture

A stock culture of *A. gossypii* was established on cotton plants with individuals obtained courtesy from Bayer AG, Leverkusen, Germany, and was kept in a climatically controlled chamber at the institute of phytopathology, University of Bonn. The stock culture was maintained on 3-week-old cotton plants, *Gossypium hirsutum* L., cv. “Caroline Queen”, at a temperature of 25±1°C, relative humidity of 60±10% and 16 hours of artificial light at an intensity of about 4000 lux. The cotton plants were usually planted in treys (60x40cm) and had been replaced with new ones fortnightly or whenever more *A. gossypii* was needed for the experiments. The leaves of the old plants were used to infest the new ones as well as to feed the predator.

2.1.1.1.2 Obtaining of individuals in the desired age

Different age groups of the *A. gossypii* individuals, 1-2- and 4-5-day-old as well as adult, were used in the experiments. The uniformly aged *A. gossypii* individuals were obtained by using rounded plastic cages, 5.5cm in diameter, and with a meshed hole in the lid to allow air exchange. The cages were filled with 0.5cm-thick-layer of 0.7% agar gel. Freshly excised cucumber leaf discs 4.5cm in diameter were placed upside down onto the agar gel layer (Fig.1). Ten adult virginoparae *A. gossypii* females were placed for 24 hours in each cage for nymphs laying. In order not to disturb the freshly laid nymphs, the adult females were gently transferred into new similarly prepared cages by using a camelhair brush. The nymphs obtained in the old cages were reared further until reaching the age desired for the experiments. The nymphs were usually kept in an incubator at a temperature of 25±1°C, relative humidity of 60+10% and a photoperiod of 16:8h (L:D).

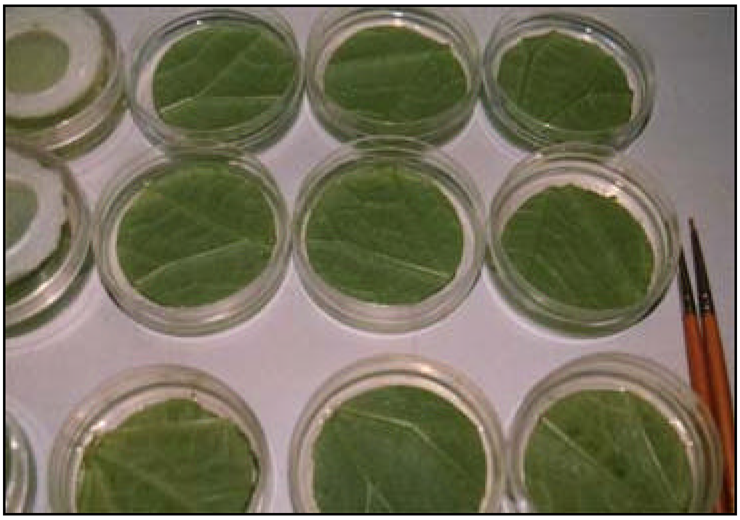

Fig. 1: Plastic cages partially filled with 0.7% agar gel and containing freshly excised cucumber leaf discs, which were used to obtain the uniformly aged *Aphis gossypii*

2.1.1.2 Rearing of *Dicyphus tamaninii*

2.1.1.2.1 Stock culture

Rearing of *D. tamaninii* was initiated with few individuals obtained courtesy from IRTA, Unitat d'Entomologia Aplicada, Barcelona, Spain. The stock culture was reared in cages (60x60x40cm) sealed with gauze from four sides in order to allow aeration. The cages were kept under controlled climatic conditions of a temperature 25±1°C, relative humidity of 60±10% and an artificial photoperiod of 16:8h (L:D) of about 4000 Lux. Tobacco plants, 3-week-old, and cucumber plants, 2-week-old, were used as a substrate for rearing and oviposition by the predatory bug. Different insect species served as prey for the rearing of *D. tamaninii.* These were *A. gossypii*, reared on cotton plants, *T. vaporariorum*, reared on tobacco plants, and eggs of *Ephestia kuehniella* ZELLER. Food was offered to the predator by placing heavily infested leaves on the substrate plants inside the cages, the moth's eggs were spread directly on the tobacco leaves. The substrate plants were usually replaced with new ones fortnightly or when needed. Before completely getting rid of the old plants, which still contains some eggs of *D. tamaninii*, they were usually kept in a separate rearing cage for about two weeks in order to give a chance for the eggs to hatch. The young predatory nymphs obtained were then gently collected by using an aspirator and transferred into the cages containing the fresh plants.

2.1.1.2.2 Obtaining of individuals in the desired age

The experiments were conducted either through the entire developmental period and longevity of *D. tamaninii* or on certain developmental stage of the predator. In order to obtain the freshly hatched *D. tamaninii* nymphs needed for the experiments, few mature *D. tamaninii* females were taken from the stock culture and then confined with a cucumber plant in a meshed cage for 24 hours for oviposition, the cucumber plant was then incubated under the same climatic conditions used in obtaining *A. gossypii* and checked twice daily for newly hatching *D. tamaninii* nymphs. The hatched nymphs were then gently collected by using an aspirator and either immediately utilised in the experiments or reared to a certain stage to be used later.

2.1.1.3 Rearing of other insects

2.1.1.3.1 Rearing of other prey insects

For the experiments on the prey preference and alternative nutritional sources for *D. tamaninii*, several arthropod pests, which usually attack vegetables in greenhouse, were reared and tested as possible preys of the polyphagous predatory bug. These were in addition to *A. gossypii*, *F. occidentalis*, *Myzus persicae* (SULZER) (Homoptera: Aphididae), *T. vaporariorum* and *Tetranychus urticae* KOCH (Acarina: Tetranychidae). All insects had been obtained from the stock cultures available at the institute for phytopathology, university of Bonn, Germany.

A colony of the Californian flower thrips *F. occidentalis* was established with few individuals, obtained from the original stock culture. The thrips were reared later on bean plants cv. Marona. Heavily infested bean plants were usually replaced by fresh ones. The rearing of the thrips took place in a climatically controlled chamber at a temperature of 25±1°C, relative humidity of 60±10% and an artificial photoperiod of 16:8h (L:D).

The green peach aphid *M. persicae* and the greenhouse whitefly *T. vaporariorum* were reared on cabbage and tobacco plants, respectively. Stock cultures of both insects were kept in acclimatised incubators under the same climatic conditions used for the rearing of *F. occidentalis*.

For rearing of the red spider mite *T. urticae*, bean leaves infested with the pest mite served as a source of inoculum for fresh bean plants, on which the spider mite was maintained. The colony was kept in an incubator under the same climatic conditions described above.

2.1.1.3.2 Rearing of other natural enemies

As *D. tamaninii* is not the only natural enemy, which would be used in a biological control program of arthropod pests, it is worthy to investigate the interaction between the predatory bug and some other natural enemies frequently used in greenhouses. Therefore, certain natural enemies were selected to be reared and used in the experiments, these are the predatory mites *Amblyseius cucumeris* OUDEMANS (Acarina: Phytoseiidae) obtained from Re-nature company GmbH in Stolpe, Germany and *Phytoseiulus persimilis* ATHIAS-HENRIOT (Acarina: Phytoseiidae) that was obtained from the stock culture available at the institute of phytopathology, as well as the solitary aphid endoparasitoid *Aphidius colemani* VIERECK (Hymenoptera: Aphidiidae), which was obtained from Koppert BV, the Netherlands.

Rearing of the two predatory mites took place in climatically controlled incubators at an alternating temperature of 25/20°C, relative humidity of 60±10% and a photoperiod of 16:8h (L:D). The predatory mite *A. cucumeris* was reared in rearing cages specially constructed for that purpose. The rearing cages constituted from a Plexiglas cage, 7.5cm x 15 cm x 4.5cm, filled with water and placed in a relatively larger cage, which was also partially filled with water. The cover of the Plexiglas cage was painted with black in order to facilitate observing the lightly coloured mites. About 50-70 predatory mites were reared on each arena. In order to avoid the escape of the mites and also provide them with water, pieces of towel paper were spread, about 1cm wide, along the edges of the Plexiglas cage and were always kept floating in the surrounding water from the other side, so that they remain permanently wet. For oviposition, transparent polyacetate pieces, 2 cm x 2 cm, were pent and placed to make a roof-shaped microhabitat, where the mites usually laid their eggs. Eggs were weekly transferred onto new rearing arena. The predator was fed on the red spider mite *T. urticae* and pollens, which were added ad libitum to the rearing arena twice weekly. *P. persimilis* was reared on bean plants, which were previously infested with the red spider mite *T. urticae* as prey.

The stock culture of the aphid endoparasitoid *A. colemani* was maintained with *A. gossypii* as a host and cucumber as a host plant. The culture was kept in a climatically controlled greenhouse compartment with a minimum temperature of 20°C and maximum temperature depended on the weather and did not exceed 30°C, the relative humidity ranged from 50 to 80% and an artificial light of 16:8h (L:D) was also provided.

2.1.2 Experimental procedures

2.1.2.1 Biology of *Dicyphus tamaninii* at different temperatures

The biology of *D. tamaninii* was comprehensively studied in the laboratory under standardised climatic conditions. The experiments on embryonic and nymphal development, mortality, longevity as well as fecundity were conducted at two constant temperatures of 25±1°C and 18±1°C, relative humidity of 60+10% and a photoperiod of 16:8h (L:D). Sex ratio, on the other hand, was studied only at 25±1°C and the same other climatic conditions described above.

The embryonic developmental period was determined by using 3-week-old cucumber plants, on which 15 adult *D. tamaninii* females were singly confined for 24 hours by clip-on-cages on the lower surface of the leaves for oviposition (Fig. 2). The females used in the experiments were 2-week-old and had access to males one week before beginning with the experiments. The leaves were previously infested with *A. gossypii* for *D. tamaninii* females to feed on. After that both *D. tamaninii* females and *A. gossypii* were removed and the cucumber plants, with the eggs inserted in them, were incubated in climatically controlled chambers and checked daily until egg hatched and the nymphs came out from the leaves. The duration between oviposition and nymph hatching was recorded.

The developmental periods of the nymphal instars were determined using 1-2- and 4-5-day-old *A. gossypii* as prey. Experiments were usually started by using freshly hatched *D. tamaninii* nymphs. The predatory nymphs were placed singly with excess number of *A. gossypii* from both age groups on cucumber leaf discs in plastic cages, which were prepared in a way similar to those used to obtain the uniformly aged aphid nymphs (Fig. 1). New plastic cages with fresh prey aphids of the desired age were daily prepared, into which *D. tamaninii* nymphs were daily transported. The old cages were daily checked for the skins of moulted nymphs and then cleaned to be used later in the experiments. Moulting and mortality of *D. tamaninii* nymphs were monitored and recorded every 12 hours. At least 14 replicates at each prey age and temperature were used.

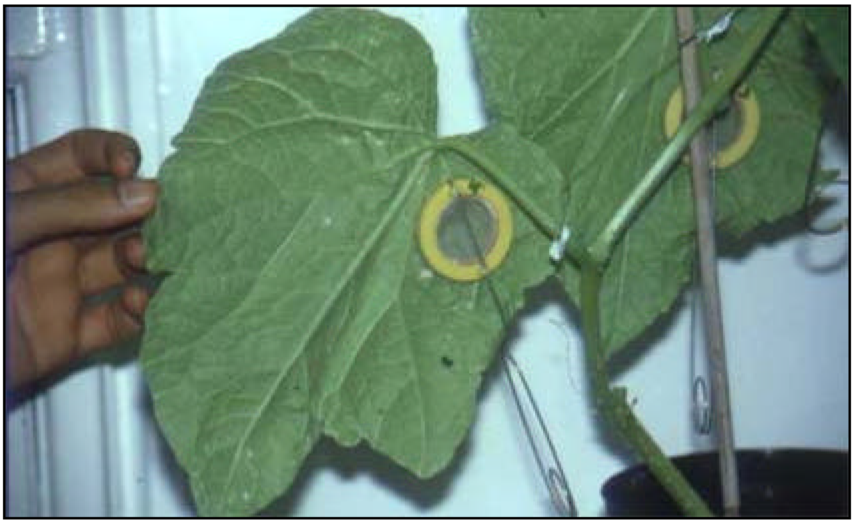

Fig. 2: Clip-on-cages used to singly confine *Dicyphus tamaninii* adult females on cucumber leaves in order to determine the embryonic developmental period

The longevity of adult *D. tamaninii* females and males was investigated with *A. gossypii* of two different age groups as prey at two different temperatures. For the experiments, last nymphal instars of *D. tamaninii* were taken from the stock culture and placed singly on cucumber leaf discs, about 8cm in diameter, which were placed in rounded cages, 11cm in diameter, with meshed holes in the lid to ensure air exchange and had been lined with a 0.5cm-thick agar gel layer. After emergence, adults were separated according to sex and offered daily an excess number of 1-2- or 4-5-day-old *A. gossypii* nymphs (>70). Females had access to males once weekly. Longevity of each single individual was recorded. At least 20 replicates were used at each temperature and prey age group of *A. gossypii*. For the purpose of comparison with the mated females, the longevity of unmated *D. tamaninii* females was studied with both prey age groups of *A. gossypii* as prey at a temperature of 25$\pm$1°C.

In order to establish the pre-oviposition, oviposition and post-oviposition period as well as the number of the total progeny produced by *D. tamaninii* females during their longevity, cucumber plants with 4 true leaves were used, where freshly emerged females had been confined singly, each with a male, on the lower surface of the leaves by a clip-on-cage for oviposition. As the predatory bug insert its eggs usually in fleshy plant tissues, care was taken that the oviposition area, on which the female was confined, always included a part of the midrib for the eggs to be inserted in. The oviposition area was always marked in order to facilitate identifying the eggs later. The clip-on-cages with females and males were daily transferred to new leaves, which were previously infested with an excess number of *A. gossypii* for *D. tamaninii* to feed on. The cucumber plants were incubated at a temperature of 25$\pm$1°C and 18$\pm$1°C. The leaves possibly containing eggs were detached from the plants after one week at 25$\pm$1°C and two weeks at 18$\pm$1°C and were then checked under binocular for the eggs inserted in them. Ten replicates were used at each temperature.

The effect of the daily prey offer on the number of eggs laid daily by *D. tamaninii* was studied at a temperature of 25±1°C and the same other climatic conditions described above. The experiments were conducted on cucumber plants, where freshly emerged *D. tamaninii* females were singly confined on the lower side of the leaves by the use of clip-on-cages. During the first 10 days of the experiment, the females were offered daily 35 individuals of 1-2-day-old *A. gossypii*. The number of prey offered was reduced to 7 *A. gossypii* individuals per day during the next week and then increased again to 35 during the following week. This alternation in the prey offer between 7 and 70 aphid/day continued over the longevity of *D. tamaninii*. The females had access to males for the first time on the 2nd day of the experiment and then once weekly. During the experiment, the females were usually transferred daily to new leaves, previously infested with the desired number of *A. gossypii*, and the old leaves were marked and checked later for the eggs inserted in them. The experiment started with 12 females and continued till the last one died.

The sex ratio of the total progeny produced by *D. tamaninii* females was determined over their entire longevity. The experiment was started with 5 freshly emerged *D. tamaninii* females, which were confined together with 5 males on a cucumber plant in a cylindrical Plexiglas cage with a meshed lid to ensure air exchange. The plant was previously infested with *A. gossypii* to serve as prey for the predatory bug, additionally *A. gossypii* was supplied on the plant every 2nd day in order to ensure food availability for the predator. Both females and males were transferred weekly onto a new plant, and the old one was further incubated and checked daily for freshly hatching *D. tamaninii* nymphs. The hatched nymphs had been daily gathered in petri dishes and fed on *A. gossypii* until reaching the adult stage where the sex was determined. Due to the long longevity of the females, sex ratio was summarised weekly.

2.1.2.2 Prey consumption by *Dicyphus tamaninii* at different temperatures

The prey consumption by *D. tamaninii* through its development as well as the entire longevity of the adult females and males was studied in the laboratory with two different age groups of *A. gossypii* as prey. All experiments on prey consumption were conducted at two different temperatures of 25±1°C and 18±1°C, relative humidity of 60±10% and a photoperiod of 16:8h (L:D).

2.1.2.2.1 Prey consumption by the nymphal instars

The laboratory experiments on the prey consumption by the nymphal instars of *D. tamaninii* were conducted on cucumber leaf discs prepared in plastic cages, similar to those used for studying the nymphal development. Soon after emerging from the egg stage, first nymphal instars (N_1) of *D. tamaninii* were confined singly in the cages and offered daily an excess number of 1-2- or 4-5-day-old *A. gossypii* individuals as prey. The number of preys offered daily was $\geq$30 for N_1-N_3 and $\geq$70 for N_4-N_5 instars. During the experiments, *D. tamaninii* nymphs were daily transferred into new cages with fresh preys, and the number of killed *A. gossypii* in the old ones was recorded. Aphids, which had been killed by the predator, were mostly emptied of fluid and easily distinguished from those still alive. At least 20 replicates were conducted with each prey age and temperature.

2.1.2.2.2 Prey consumption by the adults

The experiments on the prey consumption by the adults took place on cucumber leaf discs placed in rounded plastic cages as described in studying the biology of the predator. In the experiments, freshly emerged female and male *D. tamaninii* were kept singly in the cages and offered daily at least 70 individuals of 1-2- or 4-5-day-old *A. gossypii* individuals as prey. The females had access to males for the first time on the 2nd day after their emergence and then once weekly. The number of *A. gossypii* killed by each predatory individual was recorded daily. The experiments were continued until the death of the last predatory bug in the experiment. Fifteen replicates were used with each sex and prey age group of *A. gossypii*.

2.1.2.2.3 Prey consumption by changing prey offer

The predatory efficiency of the adult female of the predator was studied with a changing number of 1-2-day-old *A. gossypii* individuals as prey at a temperature of 25±1°C and the same other climatic conditions described above. The experiments were conducted on adult *D. tamaninii* females over a period of 3 weeks. The predatory females were placed singly on cucumber leaf discs in rounded plastic cages. Experiments were started with mated one-week-old adult *D. tamaninii* females, which were divided into 4 groups 12 individuals each, where 70, 15, 7, and 3 aphid/day were offered during the 1^{st} experimental week. The prey offer was changed then to 35 aphid/day during the 2^{nd} week, and then was switched again to 70, 15, 7 and 3 aphid/day during the 3^{rd} week of the experiment. During the experiments, the females were transferred daily to new cages containing the suitable number of *A. gossypii*, and the number of killed prey in the old ones was recorded.

2.1.2.2.4 Prey preference and alternative nutritional sources

2.1.2.2.4.1 Mixed population of *Aphis gossypii*

The prey age preference by N_3 and N_5 nymphal instars as well as adult females of *D. tamaninii* was investigated with a mixed population of *A. gossypii*. Individuals of *D. tamaninii* in the desired stage were placed singly in plastic cages and daily offered 10 individuals of each, 1-2- and 4-5-day-old as well as adult *A. gossypii* individuals together. The experiments were conducted along the entire developmental period of N_3 and N_5 instars and for three days with the adult females at a temperature of 25±1°C. In order to avoid the possibility that *D. tamaninii* might get adapted to a certain prey age of *A. gossypii*, care was taken to assure that the predatory individuals had been fed on a mixed population of *A. gossypii* over the whole period from egg hatching until the beginning of the experiments. Ten replicates were done with each developmental stage of *D. tamaninii*.

2.1.2.2.4.2 Mixed population of different prey species

A multi-choice experiment was set up in order to assess the prey preference of N_3 and N_5 instars as well as adult females of *D. tamaninii* for prey individuals belonging to 5 different species, which are *A. gossypii* (1-2-day-old), *M. persicae* (1-2-day-old), *F. occidentalis* (L_1-L_2), *T. vaporariorum* (N_2-N_3) and *T. urticae* (adults). For each predatory N_3 and N_5 instar as well as one-week-old female, 10 individuals of each prey species were simultaneously offered on cucumber leaf discs, 4.5 cm in diameter, placed in rounded plastic cages, 5.5 cm in diameter, and lined with a 0.5 cm-thick agar gel layer. The cages containing the preys and predators were incubated at a temperature of 25±1°C, relative humidity 60±10% and an artificial photoperiod of 16:8 (L:D). The number of killed preys of each species was daily determined by deducting the number of the still living individuals from their original number at the beginning of the experiments. The predators were transferred daily to new cages containing fresh preys from the 5 species. The experiments were conducted along the whole developmental period of the predatory nymphs and for 3 days with the adult females. In order to avoid a possible adaptation of the predator to a certain prey species, all the tested *D. tamaninii* individuals had been fed on a mixed population of the 5 prey species over the entire duration from their emergence until the beginning of the experiments. Ten replicates were conducted with each predatory stage tested.

2.1.2.2.4.3 Mixed population of *Tetranychus urticae*

As *T. urticae* is one of the most important arthropod pests that often attack many crops under greenhouse, it is worthy to investigate whether *D. tamaninii* can successfully feed and achieve its full development by exclusively feeding on this mite pest. Therefore, a one-choice laboratory experiment was set up, in which 20 freshly hatched *D. tamaninii* nymphs were singly placed on cucumber leaf discs in plastic cages, where excess number of *T. urticae* from different developmental stages was daily offered as prey. *T. urticae* individuals used in the experiment were obtained from a colony established on bean plants, where some leaves, heavily infested with the mite, were taken from the stock culture and the mites were brushed out of them by a mite brushing machine. The mites collected by the machine were then gently transferred into the cages by a camel hairbrush for *D. tamaninii* to prey on. The experiment was conducted under the same climatic conditions described above. Moulting and mortality of *D. tamaninii* were observed twice daily.

2.1.2.2.4.4 Alternative nutritional sources

It was observed in the studies on the biology of *D. tamaninii* that the predatory bug can also feed on the sap of the cucumber leaf discs used in the experiments. This has sparked the interest in setting up an experiment, in order to investigate how long *D. tamaninii* would survive, when certain nutritional sources are exclusively offered to the predator to feed on at a temperature of 25±1°C. Therefore, one-week-old adult *D. tamaninii* females, previously fed on *A. gossypii*, were divided into 4 groups of 15 individuals each. The individuals within each group were placed in Plexiglas cages and offered exclusively one of 4 diets, which were a mixed population of *A. gossypii*, 10% honey emulsion, a cucumber plant or left without food. The cages where checked daily for mortality in the tested individuals. Food was added or replaced whenever needed.

2.1.2.3 Effect of extreme high constant and alternating temperatures on the development, mortality and prey consumption by *Dicyphus tamaninii*

The effect of two extreme constant temperatures of 30±1°C and 35±1°C, and two alternating temperatures of 25/15±1°C (L:D) and 35/22±1°C (L:D) on the embryonic and nymphal development, mortality and prey consumption by the nymphal and adult stages of *D. tamaninii* was studied in the laboratory. The experiments were conducted in climatically controlled chambers an artificial photoperiod of 16:8h (L:D).

The experiments on the embryonic developmental period were conducted on cucumber plants, on which 15 mated 2-week-old *D. tamaninii* females were confined singly on the lower side of the leaves for 24 hours for oviposition. The egg-infested plants were then incubated at the desired temperature and checked daily for hatching nymphs.

Developmental periods of the nymphal instars were studied in rounded plastic cages containing cucumber leaf discs, on which excess number of 1-2-day-old *A. gossypii* individuals was daily offered for the predatory nymphs to feed on. The cages with the predators and preys were kept in climatically controlled chambers at the desired temperature. Moulting and mortality of the nymphs were checked twice daily. At least 15 replicates were used at each temperature.

The effect of the four temperatures was also studied on the prey consumption by *D. tamaninii* through its development from N_1 till the adult stage as well as during the first 10 days of longevity. The experiments were conducted on cucumber leaf discs in plastic cages, where excess number of 1-2-day-old *A. gossypii*, at least 30 for N_1-N_3 and 70 for N_4-N_5 and adults, was offered daily. The predator was transferred daily to new cages with fresh preys and the number of killed *A. gossypii* in the old ones was recorded. Fifteen replicates were used at each temperature.

2.1.2.4 Host plant spectrum for oviposition by *Dicyphus tamaninii*

In order to determine which host plant may be preferred by *D. tamaninii* for oviposition, about 40 N_5 instars were obtained from the stock culture and reared further in a meshed cage until reaching the adult stage. For reducing the possibility that *D. tamaninii* might get adapted to a certain plant species, the meshed cage contained six plants representing the plant species that were used in the experiments, i.e. cucumber, tobacco, tomato, beans, sweet pepper and eggplant. The adult *D. tamaninii* females and males were reared further for 7 days to give them the chance for mating. Thereafter, 15 adult females were transferred into another meshed cage containing the six different potted host plants, each with three true leaves. The adult females were provided with excess numbers of *A. gossypii* placed evenly on top of the leaves of the different host plants as prey. Feeding and checking for mortality were done daily. The plants were daily replaced with new ones. Since the eggs of *D. tamaninii* are quite difficult to be recognised directly after being inserted in the plant tissues, the old plants were kept in the rearing chamber for about 5 days in order to give the eggs enough time to develop and gain some colour, which will facilitate counting them. After that the plants were taken to the laboratory and checked under binocular for the eggs inserted in them.

2.1.2.5 Interaction of *Dicyphus tamaninii* with selected beneficial arthropods

As *D. tamaninii* is a polyphagous predator, it is worthy to study how it would interact in the presence of other natural enemies, which are usually utilised in the biological control programs of some important arthropod pests under greenhouse. Therefore, the predatory behaviour of adult

D. tamaninii females was studied when *A. cucumeris*, *P. persimilis* and *A. colemani* (in *A. gossypii* mummies) in the presence of unparasitized *A. gossypii* nymphs were simultaneously present in the experimental arena. The arena consisted of a cucumber leaf disc of a diameter 4.5 cm placed upside-down in a plastic cage, 5.5 cm in diameter, and had been lined with a 0.5 cm-thick agar gel layer. Mated one-week-old adult females of *D. tamaninii* were singly placed in the arena for 24 hours, after that the number of killed individuals was recorded. The adult predatory females used in the experiments were provided with only water for a period of 24 hours before release. Replicates, in which *A. colemani* adults emerged from the mummies during the experiment, were not considered. Ten *D. tamaninii* females were tested and the experiment was repeated for three days. Experiments were conducted under the same climatic conditions mentioned above.

2.1.2.6 Potential damage to cucumber fruits caused by *Dicyphus tamaninii* adults

The plant feeding habit of *D. tamaninii*, especially when prey offer was limited, had been observed in many experiments conducted in the present work. Feeding punctures caused by *D. tamaninii* have been occasionally observed on cucumber leaves during several experiments, but the conditions under which this occurs are currently unknown. This has stressed the necessity to set up some experiments in order to comprehensively investigate and when possible quantify the potential damage that *D. tamaninii* might cause to cucumber fruits. Two sets of experiments were designed, the first one was to study the damage caused to cucumber fruits, and the second set was devoted to investigate the leaf-fruit preference of the predator under different nutritional conditions.

2.1.2.6.1 Damage to fruits with different prey treatments

Two experiments were set up in order to investigate the potential damage, which *D. tamaninii* can cause to cucumber fruits.

The first one aimed to study how the presence or absence of leaves and prey during the experiment will affect the fruit feeding by the predator. In the experiment, groups of one-week-old adult female *D. tamaninii*, 5 individuals each, were used. Prior to the experiments, the females had access to males for 24 hours and were most likely mated. These were placed in transparent cylindrical Plexiglas cages 18 cm in diameter and 40 cm high, which have meshed lids in order to ensure air exchange. Each cage contained a puncture-free cucumber fruit, which was about 10 cm long and 3.5 cm in diameter, and one of four treatments: (1) A cucumber leaf about 20 cm^2 in area, where the leaf petiole was wrapped in wet cotton wool and maintained in a vertical position by immersing the petiole in a 30 ml glass viol filled with water. (2) A cucumber leaf and 25 individuals of 1-2-day-old *A. gossypii* which were daily added on the leaf. (3) A cucumber leaf infested daily with excess number of *A. gossypii* ($\geq$300). (4) Excess number of *E. kuehniella* eggs, which were offered on a moistened filter paper placed in a rounded plastic cage 5.5 cm in diameter. Each treatment was repeated 4 times. The cylindrical cages were closed and kept in a climatically controlled rearing chamber at a temperature of 25±1°C, relative humidity of 60±10% and an artificial photoperiod of 16:8h (L:D). After 5 days, the cucumber fruits were removed from the cages and each fruit was carefully examined for feeding punctures under binocular. The number of feeding punctures on each fruit was determined and recorded by three different observers. Replicates in which one or more *D. tamaninii* females died were discarded and repeated another time. Each *D. tamaninii* female was used only once.

The second experiment was designed to investigate the effect of the nutritional source, offered to *D. tamaninii* before starting with the experiment, on the number of feeding punctures caused by the predatory females on cucumber fruits. Therefore, the predatory females were only fed on either *A. gossypii*, cucumber plant or were starved for 24 hours prior to the experiments. After that, the females were confined singly, each with a cucumber fruit only, in cylindrical cages similar to those used in the first experiment without the presence of leaves or prey. The cylindrical cages were closed and kept in a climatically controlled rearing chamber at the same climatic conditions described above. The cucumber fruits were then removed carefully from the cylindrical cages after 24 hours and checked under binocular for feeding punctures. Thirty-six replicates were used in the experiment.

2.1.2.6.2 Leaf-fruit preference

One-week-old adult *D. tamaninii* females were employed in the experiments. These had access to males for 24 hours and were most likely mated. The females were placed individually in cylindrical Plexiglas cages, 18 cm in diameter and 40 cm high, and fitted with meshed lids for ventilation. Each cage was covered with a piece of cartoon with a black colour from all sides, in order to reduce the attractiveness of light to the predatory bugs. The cartoon cover was removed only for short periods for observation. Each cage contained a cucumber leaf about 20 cm^2 in area held vertically in a 30 ml viol filled with water, and a cucumber fruit about 10 cm long and 3.5 cm in diameter. Females were kept for 24 hours before the experiment in small plastic cages containing either: (1) “no food (starved)”, (2) cucumber leaves only “plant-fed” or (3) excess number of *A. gossypii* ($\geq$70) “Aphid-fed” on a moistened filter paper. After release into the cylindrical cages, the positions of the females were recorded at 1, 2, 6, 12, 24 and 48 hour-intervals. Experiments and observations were carried out at room temperature of 20±3°C. Twelve *D. tamaninii* females were tested.

2.2 Greenhouse experiments

Cucumber plants, variety Saladin F1, were used in the experiments. The experiments were conducted in 3 separated glass cabins (cabin I, II and III), each about 6 m^2 in area. Care was taken that the cabins be totally sealed to prevent immigration and emigration of insects. Twenty-one potted cucumber plants, each with 3 fully developed true leaves, were placed in rows of 7 in each cabin. The cucumber plants were arranged to be 30 cm distant from each other and 40 cm from the walls of the cabin. Before starting with the experiments, the two cotyledons were detached from all plants. Each plant in the three cabins was infested with 15 individuals of 3-day-old adult *A. gossypii*, where 5 adult aphids were gently transferred to the lower surface of each leaf by the use of a camel hairbrush. Mated one-week-old *D. tamaninii* females were previously reared for the release in the glass cabins. In cabin I, a total of 21 females and 21 males of *D. tamaninii*, one female and one male per plant, were released one week after the plant had been infested with *A. gossypii*. Two weeks after infesting with *A. gossypii*, the same number of *D. tamaninii* females and males was released in cabin II. No predators were released in cabin III, which served as a control treatment. Three cucumber plants were randomly taken from each cabin starting from the first week after infestation and then once weekly. These were transferred to the laboratory in order to determine the number of adult and nymphal stages of *A. gossypii* individuals as well as the number of *D. tamaninii* nymphs on the whole plant. The number of *D. tamaninii* adults existing on the selected plants was recorded on the spot before transferring the plants to the laboratory, while the number of the *D. tamaninii* eggs inserted in each plant was determined by singly incubating the plants till all eggs hatched, where the number of emerging nymphs was recorded. The experiment continued 7 weeks till all plants were tested.

2.3 Statistical Analysis

Data were first tested for normal distribution (MURDA 1958) and homogeneity of variance utilising the Barlett-test (KÖHLER et al. 1984). The data sets that had not fulfilled the assumption of normal distribution were transformed using the Box-Cox formula: $Y = X^2 - 1/$*ë* for *ë*   0, $Y = \ln X$ for *ë* = 0, where Y is the transformed value, X the untransformed value, and $0 \leq$ *ë* ≤ 1 (ANONYMOUS 1996).

For the statistical comparison among several means, one- or multifactor-analysis of variance was conducted. Significant differences were determined utilising the Duncan's Multiple Range Test at $p \leq 5\%$ (RENNER 1981). When comparing between only two means T-Test was conducted.

3 RESULTS

3.1 Laboratory experiments

3.1.1 Biology of *Dicyphus tamaninii* at different temperatures

This part deals with the results of the laboratory experiments, which had been conducted to study the embryonic and nymphal development, mortality, longevity and fecundity of *D. tamaninii* at two different temperatures of 25±1°C and 18±1°C, relative humidity of 60±10% and a photoperiod of 16:8h (L:D) with two different age groups of *A. gossypii* as prey. The sex ratio of the progeny produced by *D. tamaninii* females over their entire longevity was studied only at a temperature of 25±1°C and the same other climatic conditions.

3.1.1.1 Embryonic and nymphal development

As *D. tamaninii* inserts its eggs in the plant tissue, only the freshly hatched nymphs coming out of the leaves were to be monitored, which makes the determination of the embryonic developmental period of each sex very difficult. Therefore, it was pooled and expressed for both sexes together. As to be seen in table 1, the nymphs emerged 12.5 days after oviposition at 25±1°C, while at 18±1°C the embryonic development lasted with 22.1 days significantly longer.

Tab. 1: Average embryonic developmental periods of *Dicyphus tamaninii* at two different temperatures

Temperature °C	Replicates	Embryonic developmental period (days)	
		Average ± SE	Min. – Max.
25±1	26	12.5 ± 0.2 a	11 - 14
18±1	25	22.1 ± 0.4 b	19 - 25

Values with different letters are significantly different at p≤5% (T-test)

Results showed that *D. tamaninii* was able to develop and reach the adult stage at both temperatures of 25±1°C and 18±1°C tested. Both age groups of *A. gossypii* nymphs used, 1-2- and 4-5-day-old were found to be suitable preys for *D. tamaninii* to develop. The nymphal development of *D. tamaninii* included 5 nymphal instars (Fig. 3), whose average developmental period at both temperatures and prey age groups are shown in table 2 and 3.

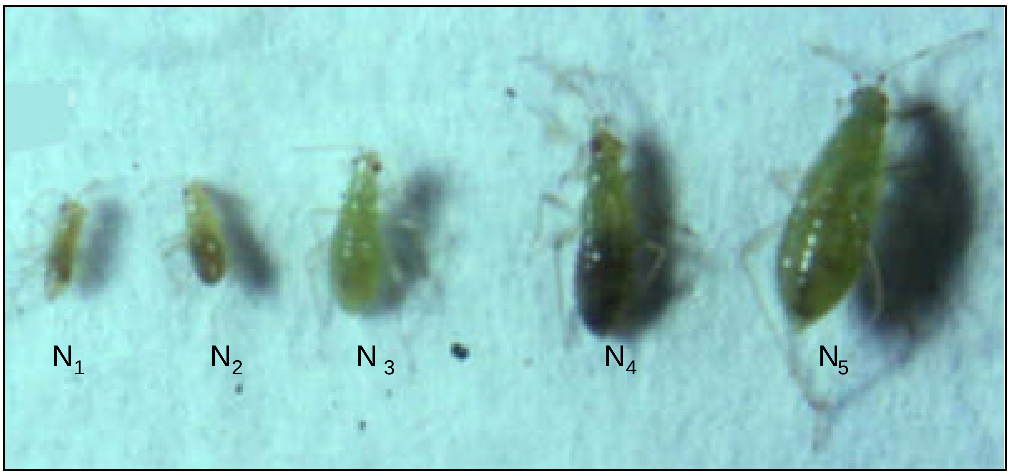

Fig. 3: The 5 nymphal instars of *Dicyphus tamaninii*

Tab. 2: Average nymphal developmental periods of *Dicyphus tamaninii* at 25±1°C with two different age groups of *Aphis gossypii* offered ad libitum on cucumber leaf discs as prey

Prey age (days)	Replicates	Sex	Nymphal developmental period (days)				
			N_1	N_2	N_3	N_4	N_5
1-2	7	**&&**	4.2	3.2	3.5	4.2	6.2
	10	**%%**	4.1	3.6	3.8	3.9	6.4
4-5	7	**&&**	6.4	5.5	5.2	5.3	7.5
	8	**%%**	6.1	5.2	4.9	5.3	7.3

The nymphal development at 25±1°C, with 1-2-day-old aphids as prey is represented in table 2. Among the 5 nymphal instars of both sexes, N_5 was longest with both prey age groups used, where it lasted with 1-2-day-old *A. gossypii* 6.2 (**&&**), and 6.4 (**%%**) days, while with 4-5-day-old prey it was 7.5 (**&&**) and 7.3 (**%%**) days. The development from N_1 to the adult stage lasted in average 21.3 (**&&**), 21.8 (**%%**) days and 29.9 (**&&**), 28.8 (**%%**) days when 1-2- and 4-5-day-old *A. gossypii* were offered as prey, respectively. Feeding on 4-5-day old *A. gossypii* had prolonged the nymphal development of *D. tamaninii*. No significant differences were found in the developmental periods between the females and males.

Table 3 shows the average nymphal developmental periods of the predator at 18±1°C with two different age groups of *A. gossypii* offered. N_5 lasted longer than all other nymphal instars with both prey age groups, where it was with 1-2-day-old *A. gossypii* in average 13.0 (**&&**), 13.9 (**%%**) days and with 4-5-day-old aphids 13.3 (**&&**) 14.3 (**%%**) days. When 1-2-day-old *A. gossypii* were offered the predatory bug reached the adult stage with 44.4 (**&&**), 45.3 (**%%**) days after egg hatching in a significantly shorter period than with 4-5-day-day-old aphids where it needed 48.7 (**&&**) 51.9 (**%%**) days. With both prey age groups offered, there were no significant differences between the developmental periods of the female and male *D. tamaninii*.

Tab. 3: Average nymphal developmental periods of *Dicyphus tamaninii* at 18±1°C with two different age groups of *Aphis gossypii* as prey offered ad libitum on cucumber leaf discs

Prey age (days)	Replicates	Sex	Nymphal developmental period (days)				
			N_1	N_2	N_3	N_4	N_5
1-2	8	**&&**	8.1	7.1	7.4	8.8	13.0
	9	**%%**	7.2	7.3	8.1	8.8	13.9
4-5	7	**&&**	10.1	6.9	9.1	9.3	13.3
	7	**%%**	10.9	8	9.6	9.1	14.3

Figure 4 represents the average total developmental periods of *D. tamaninii* from the egg to the adult stage at the temperatures 25±1°C and 18±1°C with two different age groups of *A. gossypii* as prey. The total developmental period was at 25±1°C in average 33.6 (**&&**), 34.6 (**%%**) days and 42.3 (**&&**), 41.1 (**%%**) days, while at 18±1°C it valued in average 66.1 (**&&**), 67.2 (**%%**) days and 70.9 (**&&**), 73.9 (**%%**) days, when the predator was fed on 1-2- and 4-5-day-old *A. gossypii*, respectively.

As to be seen from the results, within the same temperature, *D. tamaninii* completed its development in a significantly shorter duration when fed on 1-2- rather than 4-5-day-old *A. gossypii*. There were considerably significant differences between the two temperatures tested.

When the predator was fed on the same prey age, decreasing the temperature from 25±1°C to 18±1°C had significantly prolonged the developmental period of the predator. Within the same temperature and prey age, there were no significant differences between the total developmental periods of *D. tamaninii* females and males.

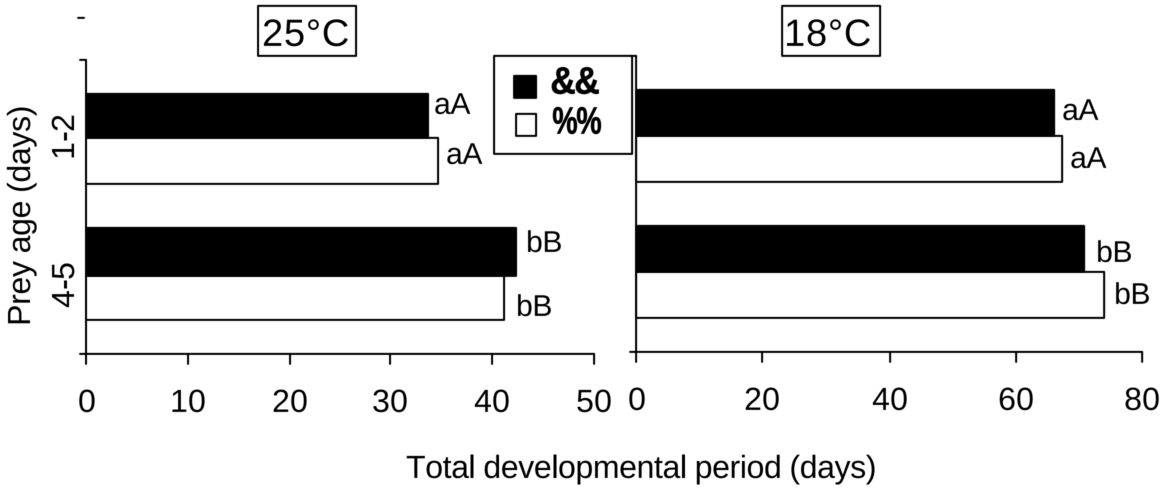

Fig. 4: Average total developmental period of *Dicyphus tamaninii* from the egg till the adult stage by feeding on 1-2- and 4-5-day-old *Aphis gossypii* as prey at different temperatures. [Bars with different small letters are significantly different within the same prey age and temperature. Bars with different capital letters are significantly different within the same sex and temperature at $p \leq 0.05$ (T-test)]

3.1.1.2 Mortality

The mortality of *D. tamaninii* during development at the temperatures 25±1°C and 18±1°C is shown in figure 5. Mortality occurred mainly in the first two nymphal instars, particularly during moulting. The total mortality during development at both temperatures was considerably lower when the predatory nymphs were fed on 1-2- rather than 4-5-day-old *A. gossypii*. The total mortality during development from N_1 to adult stage at 25±1°C valued 20% and 25%, while at 18±1°C it was 11% and 21% with 1-2- and 4-5-day-old *A. gossypii* as prey, respectively.

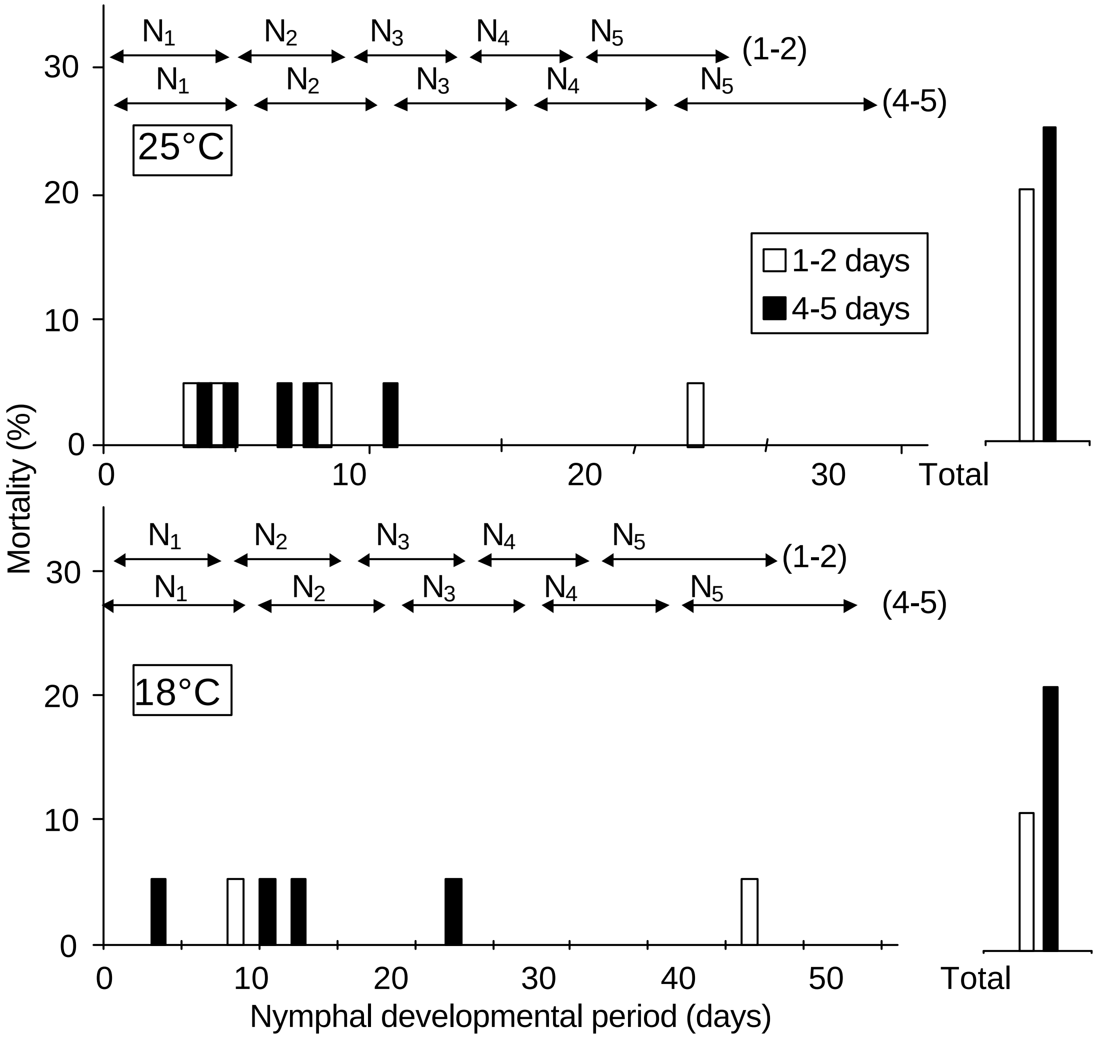

Fig. 5: Percentage mortality of *Dicyphus tamaninii* during nymphal development by feeding on 1-2- and 4-5-day-old *Aphis gossypii* on cucumber leaves at different temperatures

3.1.1.3 Longevity

The longevity of mated *D. tamaninii* was investigated on adult females and males with 1-2- and 4-5-day-old *A. gossypii* as prey at the two standardised constant temperatures of 25±1°C and 18±1°C. The longevity of unmated *D. tamaninii* females was also studied by feeding on 1-2-day-old aphids at 25±1°C. As to be seen in table 4, no significant difference was found in the longevity of the mated and unmated females where it was 29.0 and 31.8 days, respectively.

Tab. 4: Average longevity of mated and unmated females of *Dicyphus tamaninii* by feeding on 1-2- day-old *Aphis gossypii* on cucumber leaves at 25±1°C

Females	Replicates	Longevity ± SE (days)	
		Average ± SE	Min. – Max.
Mated	9	29.0 ± 7.4 a	12 - 50
Unmated	10	31.8 ± 5.5 a	10 - 61

Values with different letters are significantly different at p≤5% (T-test)

Longevity of adult mated females and males of *D. tamaninii* at both temperatures and prey age groups tested is presented in figure 6. Results showed that the longevity of *D. tamaninii* varied widely according to the temperature and prey age used, where it valued at 25±1°C in average 29.0 (**&&**), 26.3 (**%%**) days and 44.1 (**&&**), 31.4 (**%%**) days, while at 18±1°C it was in average 80.7 (**&&**), 65.5 (**%%**) days and 62.3 (**&&**), 65.4 (**%%**) days, when the predator was fed on 1-2- and 4-5-day-old *A. gossypii* individuals, respectively. In general, *D. tamaninii* adults lived shorter at 25±1°C than at 18±1°C. Longevity varied according to the prey age group of *A. gossypii* offered, although statistical analysis had showed that such variations were not significant. Temperature had a pronounced effect on the longevity of both the females and males of the predatory bug, where decreasing the temperature from 25±1°C to 18±1°C had significantly prolonged the longevity with both prey age groups offered.

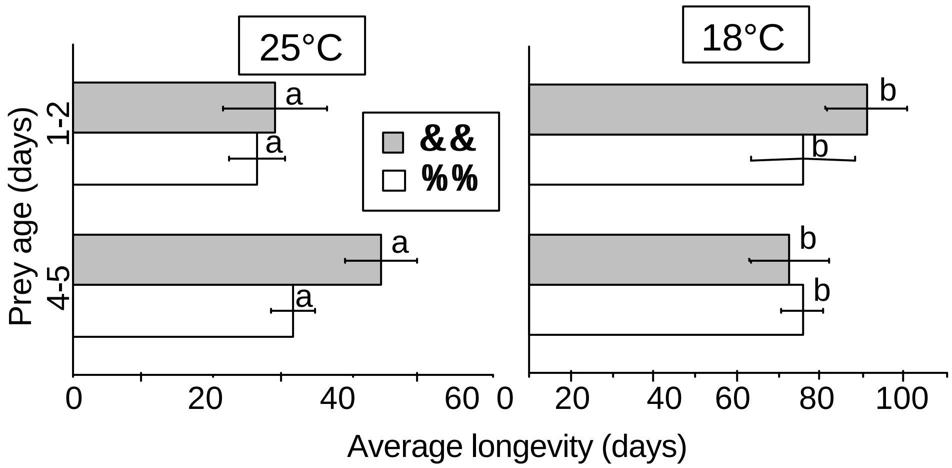

Fig. 6: Average longevity of *Dicyphus tamaninii* females and males by feeding on 1-2- and 4-5- day-old *Aphis gossypii* on cucumber leaves at different temperatures. [Different letters indicate significant differences at the two temperatures within the same sex and prey age at p≤5% (T-Test)]

Fig. 7 shows the percentage survival rate of female and male *D. tamaninii* with 1-2- and 4-5-day-old *A. gossypii* as prey at two different temperatures. At 25±1°C, with 1-2-day-old prey, at least 50% of the experimental predators were still alive 22 (**&&**), 29 (**%%**) days after adult emergence, while with 4-5-day-old aphids, after 41 (**&&**), 34 (**%%**) days. At a temperature of 18±1°C the percentage survival rate was considerably higher, where 50% of the predators were still alive 67 (**&&**), 60 (**%%**) days and 78 (**&&**), 67 (**%%**) days after their emergence with 1-2- and 4-5-day-old *A. gossypii*, respectively.

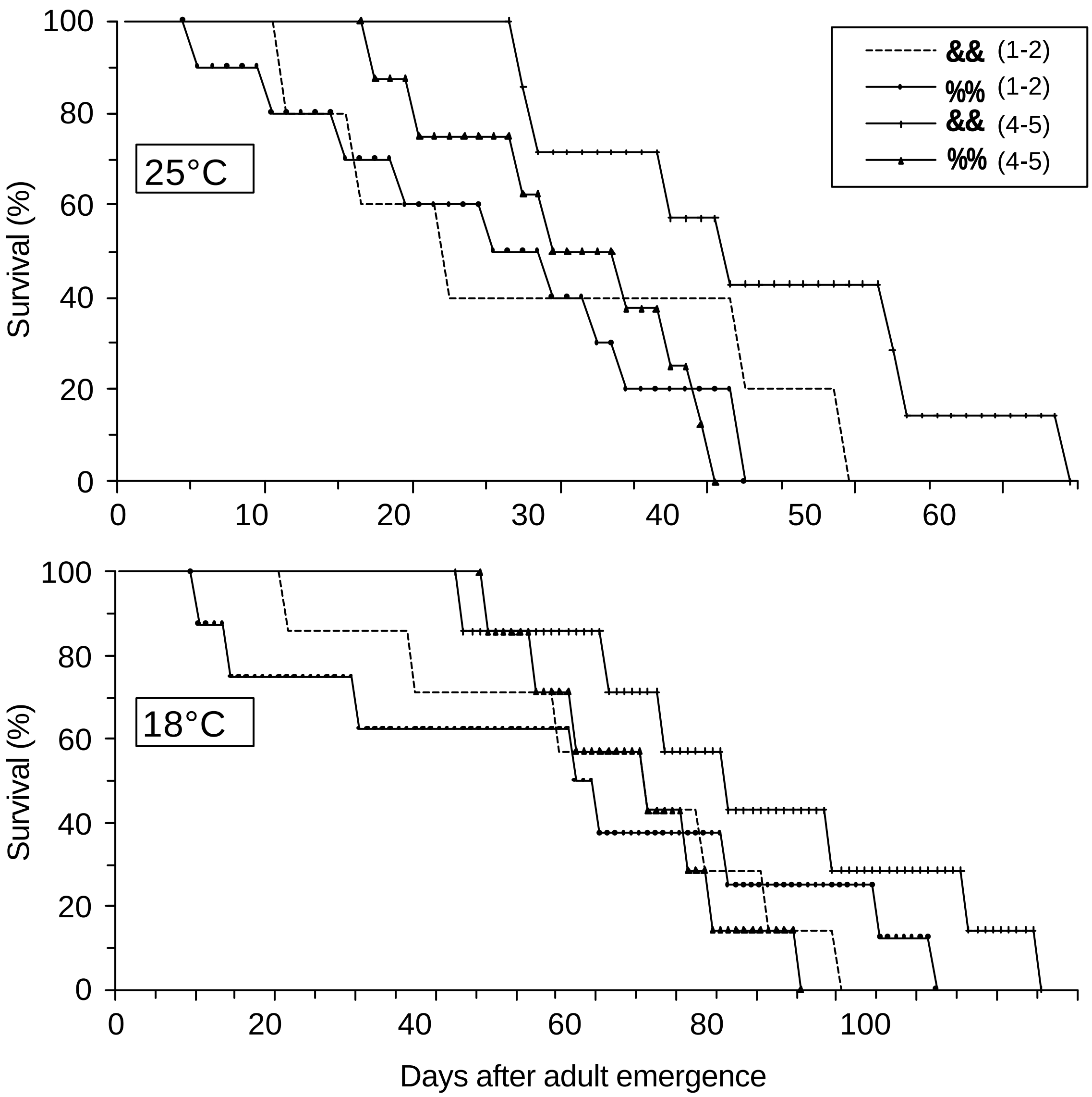

Fig. 7: Percentage survival rate of *Dicyphus tamaninii* females and males by feeding on 1-2- and 4-5-day-old *Aphis gossypii* as prey on cucumber leaves at different temperatures

3.1.1.4 Fecundity

3.1.1.4.1 Fecundity at different constant temperatures

As parameters for the fecundity of *D. tamaninii*, the pre-oviposition-, oviposition- and post-oviposition periods as well as the total number of laid eggs/**&** were studied at two different temperatures of 25±1°C and 18±1°C. Cucumber plants were used as the substrate plant for oviposition and the predatory nymphs were fed on mixed population of *A. gossypii*. As table 5 shows, *D. tamaninii* began with oviposition 4-6 days after adult emergence at 25±1°C, where a female laid in average a total of 67.8 eggs over an oviposition period of 19-59 days. The post-oviposition period at 25±1°C showed also considerable fluctuations where it ranged from 5-17 days. Reducing the temperature from 25±1°C to 18±1°C had resulted in a significant reduction in the number of laid eggs/**&**, where a female laid in average a total of 11.0 eggs over an oviposition period ranging from 21-57 days. Furthermore, the pre-oviposition period at 18±1°C with 10-15 days was significantly longer than that at 25±1°C. The post-oviposition period at 18±1°C varied widely and ranged from 6-58 days.

Tab. 5: Pre-oviposition, oviposition and post-oviposition periods as well as the total number of eggs laid by *Dicyphus tamaninii* during the oviposition period at two different temperatures with a mixed population of *Aphis gossypii* offered ad libitum as prey on cucumber leaves

Temperature °C	Replicates	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Total number of laid eggs	
		Min.-Max	Min.-Max	Min.-Max.	Average	Min.-Max.
25±1	10	4-6	19-59	5-17	67.8 a	30-83
18±1	10	10-15	21-57	6 - 58	11.0 b	5-19

Values with different letters are significantly different at p≤5% (T-test)

The fecundity of *D. tamaninii* females at the temperatures 25±1°C and 18±1°C is represented in figure 8. At 25±1°C females started laying eggs on the 4th day after their emergence, where they laid in average 1 egg/**&**. The average number of daily laid eggs fluctuated hereafter and reached a maximum of 2.9 eggs/**&** on the 37th day. Starting from the 38th day the number of daily laid eggs began to decrease gradually till it reached zero on the 63rd day, after that no eggs were laid by the females. The first female died at 25±1°C on the 31st day while the last one died on the 70th day. At 18±1°C adult females began oviposition 10 days after emergence, where the daily number of laid eggs was in average 0.3 on the 10th day and fluctuated hereafter to reach a maximum of 0.55 eggs/**&** on the 44th day. After that the average daily number of laid eggs began to decrease gradually till it approached zero on the 69th day. During the experiment at 18±1°C the first female died on the 44th day, while the last one died on the 111th day after adult emergence.

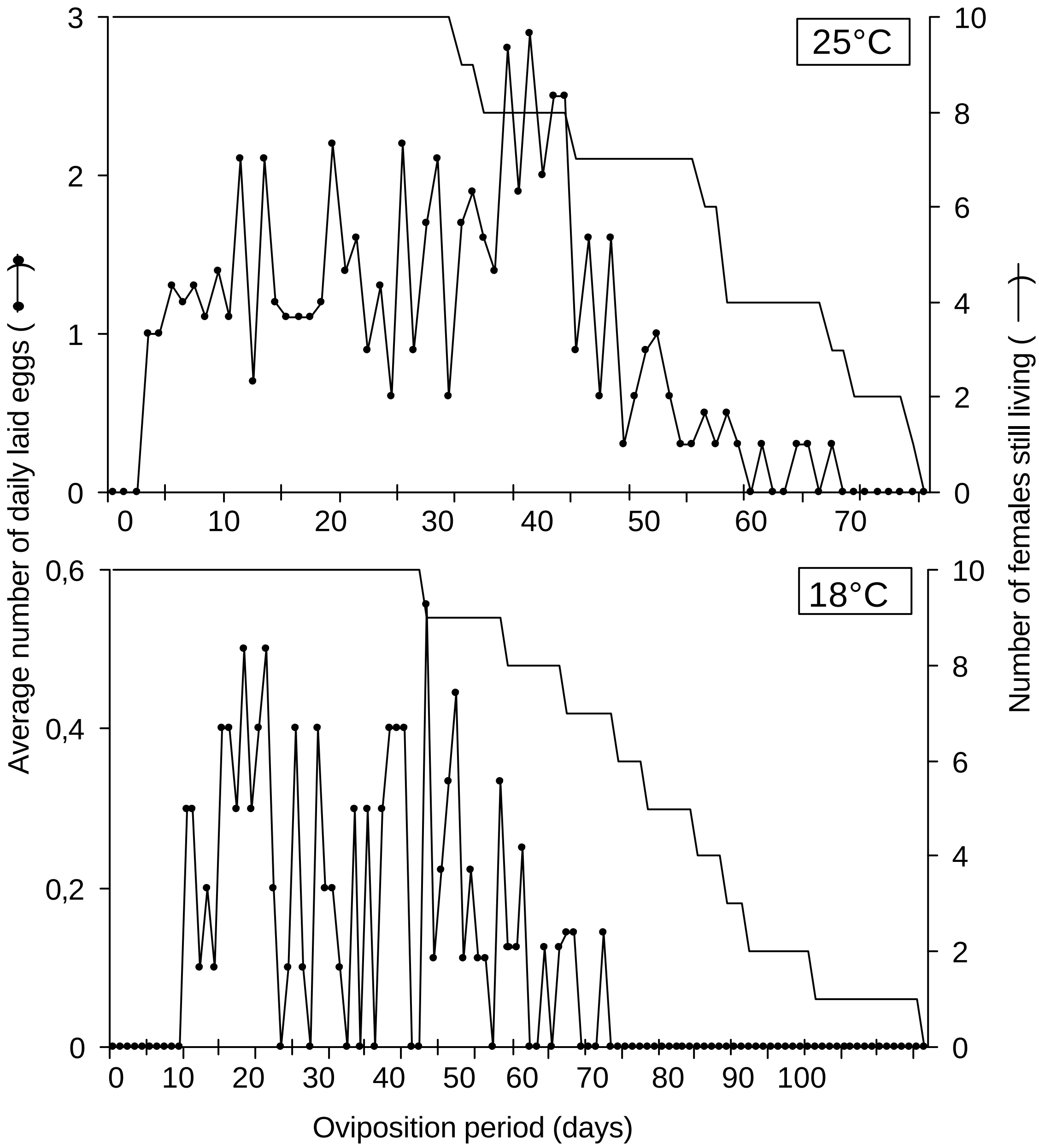

Fig. 8: Average number of daily laid eggs by *Dicyphus tamaninii* females during the oviposition period by feeding on *Aphis gossypii* on cucumber leaves at different temperatures

3.1.1.4.2 Fecundity by changing prey offer

The ability of a natural enemy to successfully reproduce with fluctuating prey availability is an important factor, which determines its suitability in biological control programs. Therefore, the pre-oviposition, oviposition and post-oviposition period as well as the number of eggs laid daily by *D. tamaninii* females were investigated at 25±1°C with a fluctuating number of 1-2-day-old *A. gossypii* individuals as prey. As table 6 shows, with a fluctuating prey offer *A. gossypii*, *D. tamaninii* started laying eggs 3-7 days after their emergence. The females laid in total 21-51 egg/**&** over a period ranging from 15-57 days. The post-oviposition period ranged from 2-10 days.

Tab. 6: Pre-oviposition, oviposition and post-oviposition periods as well as the total number of eggs laid by *Dicyphus tamaninii* females during longevity when fed on a fluctuating number of 1-2-day-old *Aphis gossypii* offered as prey on cucumber leaves at 25±1°C

Temperature °C	Replicates	Pre-oviposition period (days) Min. - Max	Oviposition period (days) Min. - Max	Post-oviposition period (days) Min. - Max.	Total number of laid eggs	
					Avr.	Min. - Max.
25±1	12	3 - 7	15 - 57	2 - 10	37	21 - 51

Figure 9 represents the number of eggs laid daily by *D. tamaninii* females over their entire longevity with fluctuating prey offer of *A. gossypii* individuals. In general, the number of daily laid eggs had considerably increased by increasing the number of *A. gossypii* offered. During the first 10 days after adult emergence, where the daily prey offer was 35 aphid/**&**, one *D. tamaninii* female laid in average up to 2 egg/day. During the following week, where the daily prey offer was reduced to 7 aphid/**&**, the average number of eggs laid per day fell to less than 1 egg/**&**. Starting from the 18th till the 26th day where 35 aphid/day/**&** were offered again, the oviposition activity of the predatory females became with up to 2 egg/day/**&** considerably higher. This fell another time to less than an average of 0.7 egg/day/**&** in the period between the 27th and 33rd day, where the prey offer had been reduced to 7 aphid/day/**&**. Increasing the prey offer to 70 aphid/day/**&** had resulted in a remarkable increase in the number of laid eggs in the period between the 34th and 41st day where up to 2.9 egg/**&** were laid daily. Starting from the 47th day, increasing the daily prey offer either to 35 or even to 70 had not resulted in a considerable increase in the number of daily laid eggs.

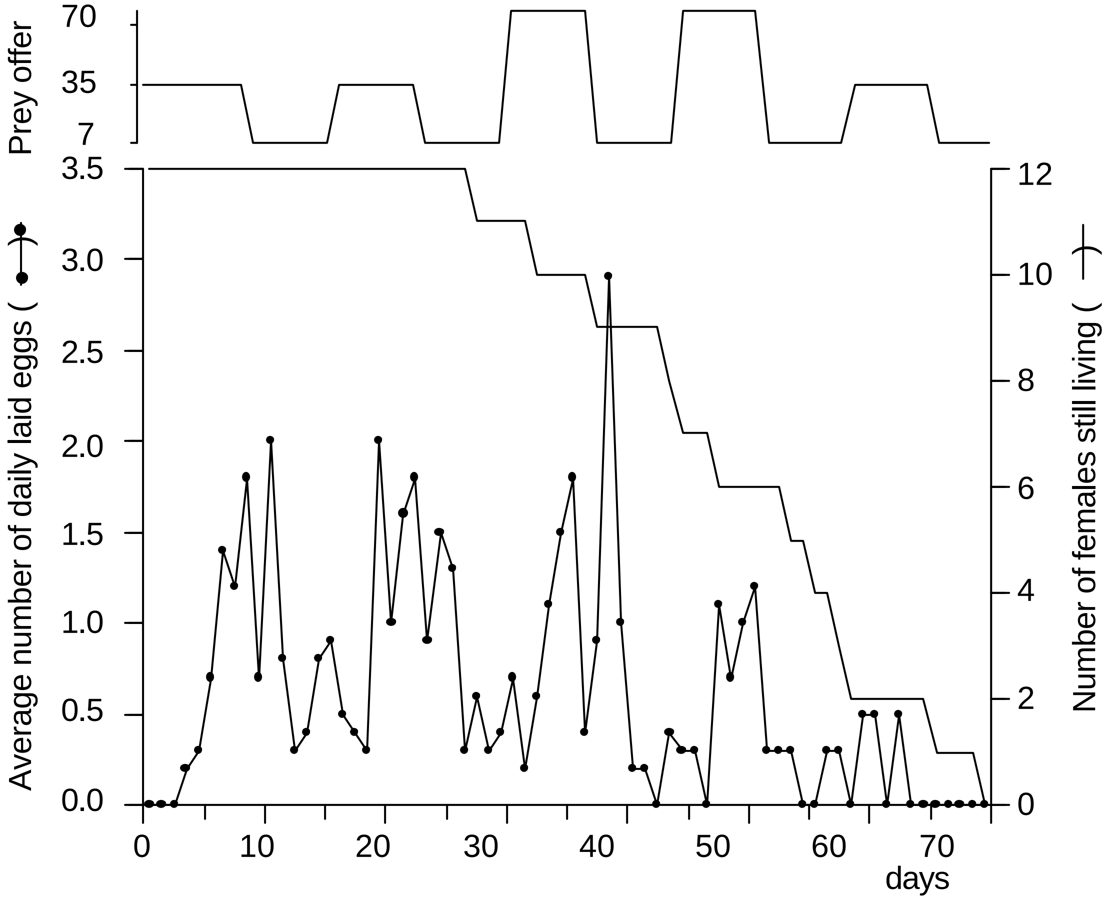

Fig. 9: Average number of daily laid eggs by *Dicyphus tamaninii* females during the oviposition period by feeding on a fluctuating number of 1-2-day-old *Aphis gossypii* offered as prey on cucumber leaves at 25±1°C

3.1.1.5 Sex ratio

The percentage sex ratio of the progeny produced by 5 mated one-week-old *D. tamaninii* females over their longevity was determined with mixed population of *A. gossypii* as prey at 25±1°C. Due to the relatively long longevity of the predator, the sex ratio was summarised weekly. Figure 10 shows the percentage sex ratio of the progeny of 5 *D. tamaninii* females. The percentage of females in the total progeny was 56% in the 1st week, which was also the highest female percentage during the longevity of the females. As the parent females aged, the percentage of females in the progeny decreased to 54% in the 2nd week, 43% in the 3rd week and it continued to decline until no females were produced in 9th week.

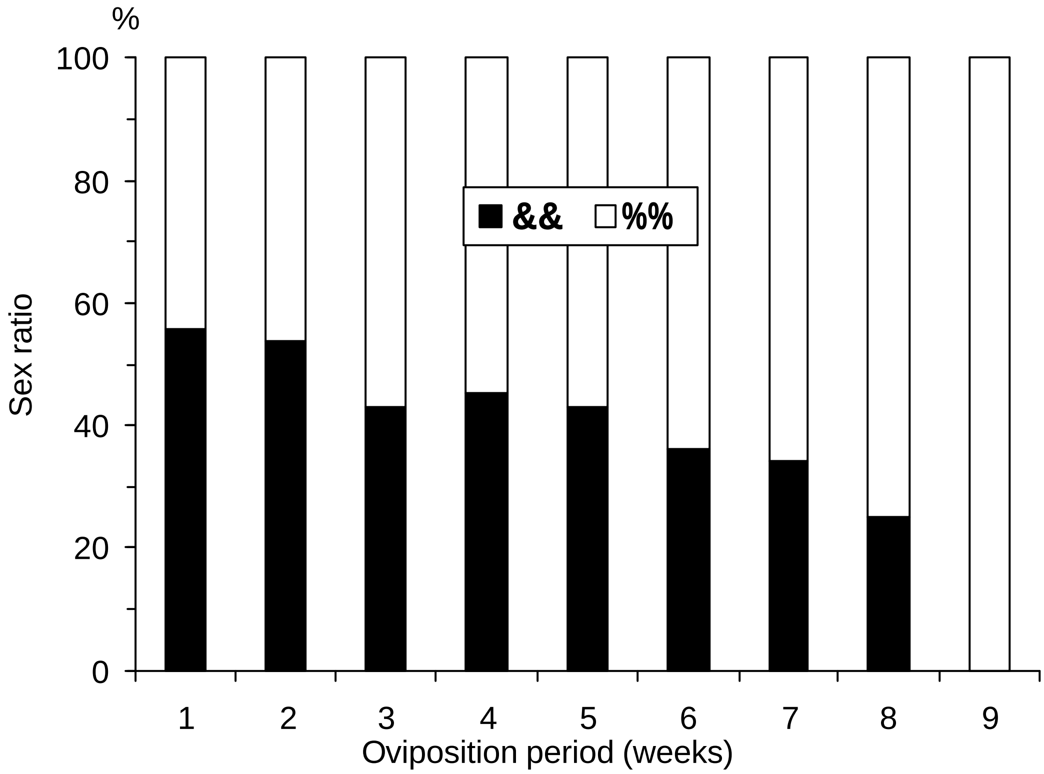

Fig. 10: Weekly summarised percentage sex ratio of the progeny produced by 5 *Dicyphus tamaninii* females by feeding on *Aphis gossypii* as prey on cucumber leaves

3.1.2 Prey consumption by *Dicyphus tamaninii* at different temperatures

3.1.2.1 Prey consumption by the nymphal instars

The prey consumption by *D. tamaninii* during its development was studied in the laboratory at a temperature of 25±1°C and 18±1°C with 1-2- and 4-5-day-old *A. gossypii* as prey. With both prey age groups offered, *D. tamaninii* nymphs started feeding only few hours after egg hatching. Figure 11 shows the average total prey consumption by each one of the 5 nymphal instars of *D. tamaninii* at both temperatures tested.

At 25±1°C, when 1-2-day-old aphids were offered, the first nymphal instar N_1 consumed in average a total of 16.9 (**&&**), 17.3 (**%%**) aphids, while with 4-5-day-old preys it consumed 8.7 (**&&**), 7.8 (**%%**) aphids. The prey consumption continued to increase with development till it was highest in N_5 instar where it valued in average a total of 173.4 (**&&**), 150.1 (**%%**) aphids and 82.9 (**&&**), 67.9 (**%%**) with 1-2- and 4-5-day-old *A. gossypii*, respectively.

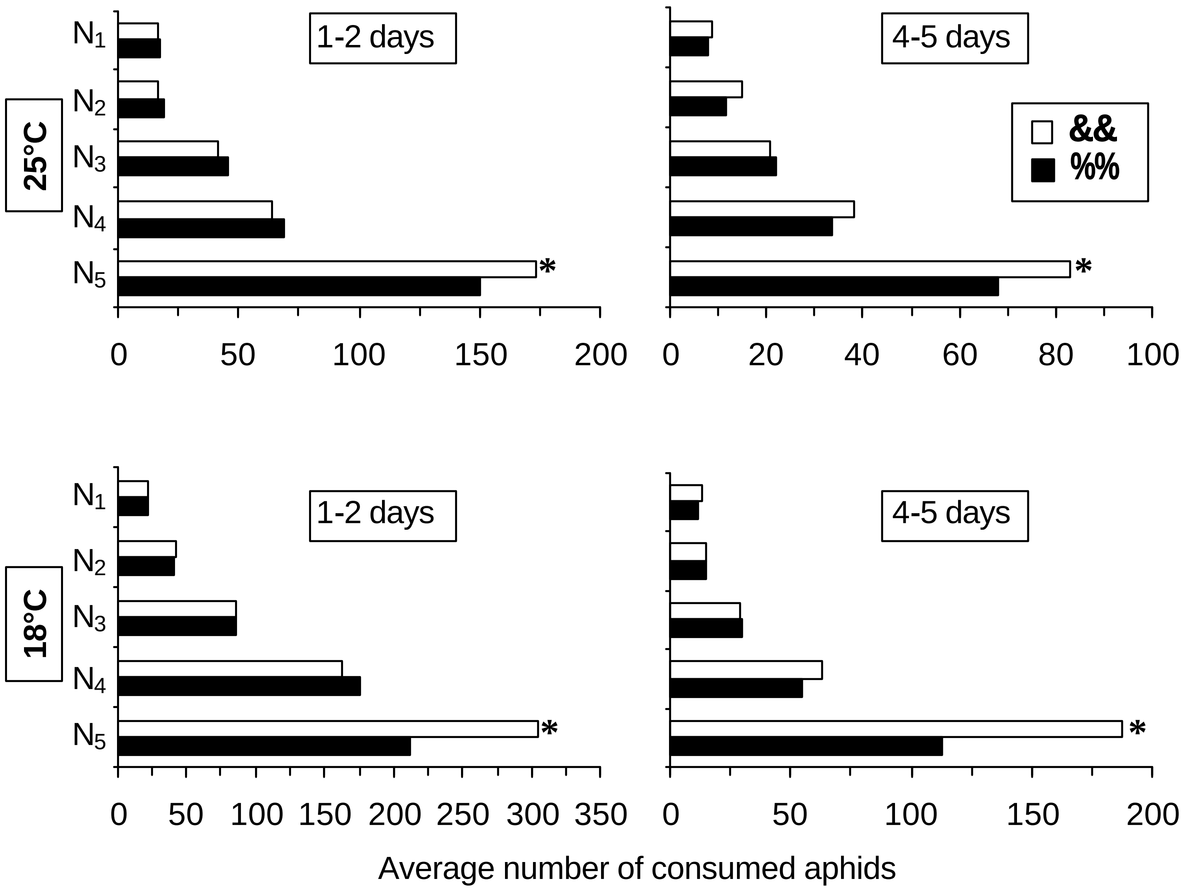

Fig. 11: Average number of *Aphis gossypii* of two different age groups consumed daily by *Dicyphus tamaninii* over its development at two different temperatures. (*) indicates significant differences between **&&** and **%%** within the same temperature and prey age group at p≤5%

At a temperature of 18±1°C, the average total prey consumption by N_1 was with 1-2-day-old *A. gossypii* 22.2 (**&&**), 21.2 (**%%**) and with 4-5-day-old aphids 13.4 (**&&**), 11.6 (**%%**), then it increased with development where it reached 304.9 (**&&**), 210.9 (**%%**) aphids and 187.8 (**&&**), 112.2 (**%%**) aphids from both age groups, respectively. At both temperatures and prey age groups tested, the prey consumption by female and male nymphs of *D. tamaninii* in the first 4 nymphal instars showed no significant differences. However, female N_5 consumed significantly more *A. gossypii* from both age groups than the male.

Figure 12 shows the average daily prey consumption of *D. tamaninii* nymphs at 25±1°C with 1-2- and 4-5-day-old *A. gossypii* as prey. The average daily prey consumption with 1-2-day-old *A. gossypii* was 4.0 (**&&**) and 4.6 (**%%**) aphids on the 1^{st} day after hatching and increased gradually to reach a peak of 34.8 (**&&**) and 34.4 (**%%**) aphids by N_5 on the 17^{th} day. When 4-5-day-old *A. gossypii* individuals were offered, the daily prey consumption valued in average 1.0 (**&&**) and 0.8 (**%%**) on the 1^{st} day and increased to a peak of 13.6 (**&&**) and 10.3 (**%%**) on the 26^{th} and 23^{rd} day, respectively. It is to be noticed, that *D. tamaninii* nymphs ceased feeding few hours ahead of each moulting to the next nymphal instar. In total, *D. tamaninii* consumed during its development from N_1 to the adult stage significantly more 1-2- rather than 4-5-day-old *A. gossypii*, where it consumed in average 313 (**&&**), 301 (**%%**) aphids and 166 (**&&**), 143 (**%%**) aphids of both age groups, respectively.

At 18±1°C (Fig. 13), when 1-2-day-old *A. gossypii* were offered, the average daily prey consumption by *D. tamaninii* nymphs was 4.0 (**&&**) and 4.3 (**%%**) aphids on the 1^{st} day after egg hatching and increased continuously with the nymphal development to reach a peak of 37.1 (**&&**) and 31.5 (**%%**) aphids on the 39^{th} and 40^{th} day, respectively. On the other hand, when 4-5-day-old preys were offered, the average daily prey consumption started with 0.9 (**&&**) and 0.4 (**%%**) on the 1^{st} day and reached 20.6 (**&&**) and 11.8 (**%%**) on the 41^{st} day. The average total prey consumption during the developmental period valued with 1-2-day-old prey 618 (**&&**), 533 (**%%**) aphids while with 4-5-day-old prey it was 309 (**&&**), 224 (**%%**) aphids. Such differences in the prey consumption were found to be significant.

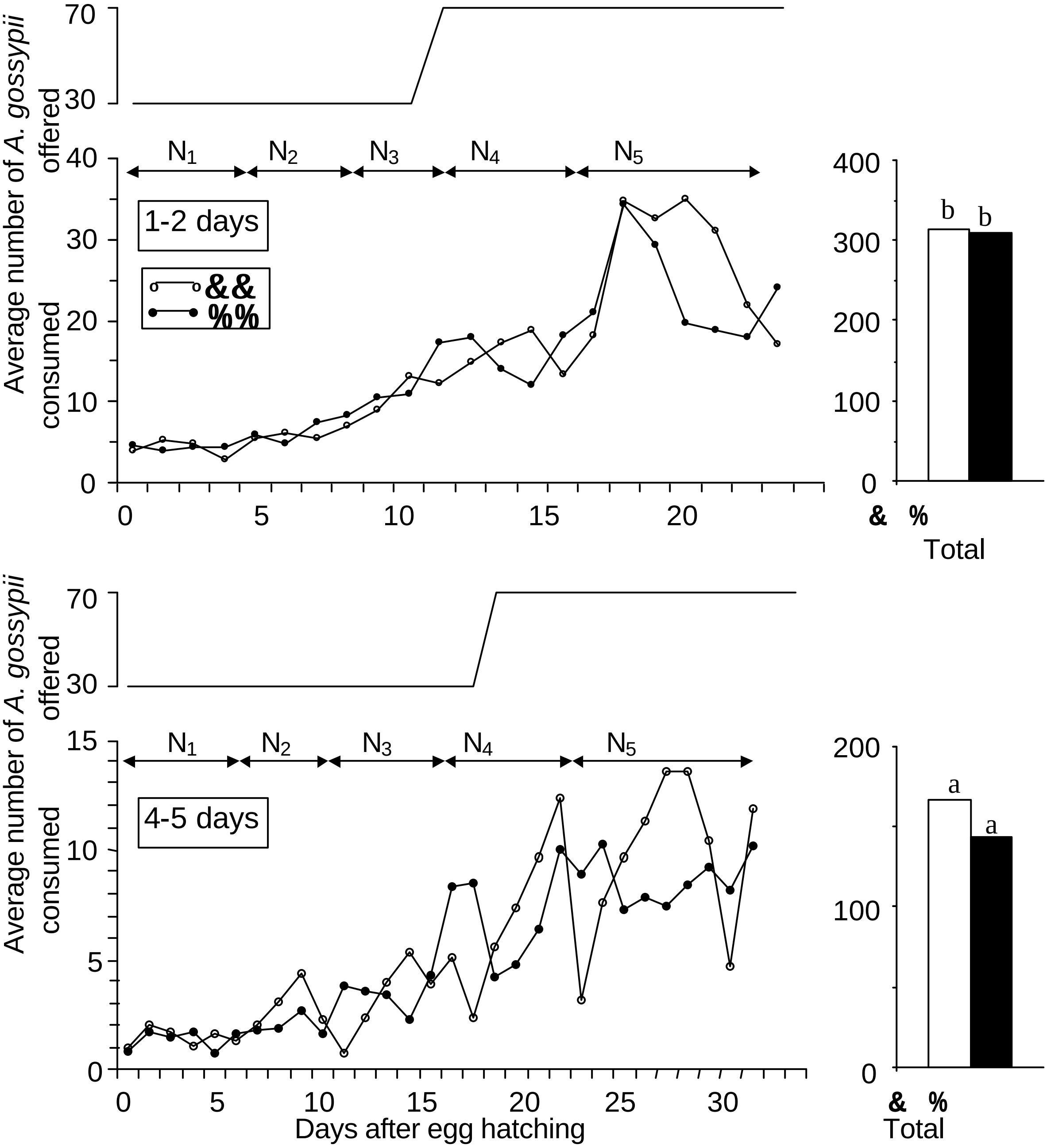

Fig. 12: Average daily prey consumption by female and male *Dicyphus tamaninii* during development on 1-2- and 4-5-day-old *Aphis gossypii* individuals offered on cucumber leaves at 25±1°C. [Bars with different letters are significantly different at $p \leq 5\%$ (Two-factor analysis of variance)]

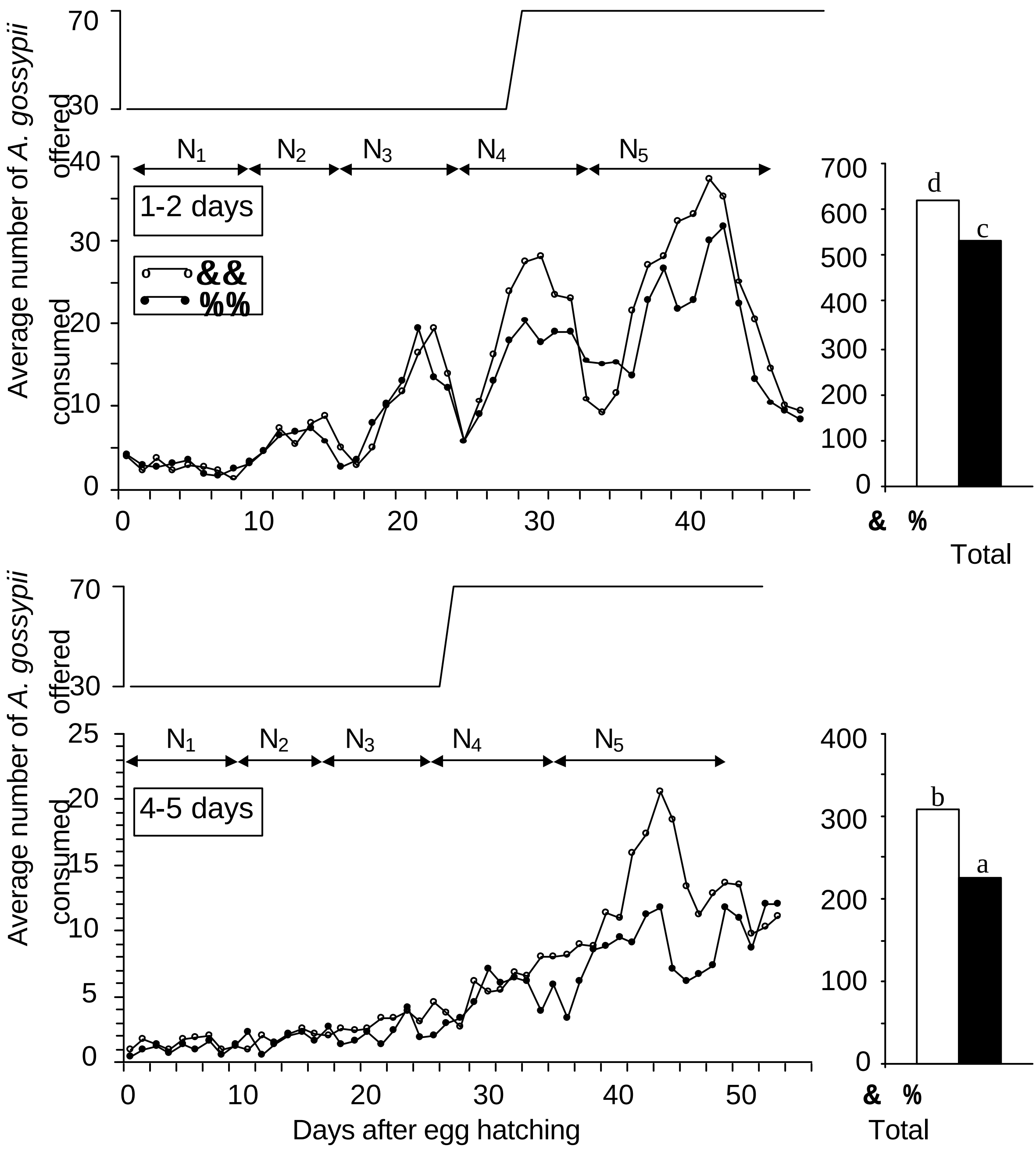

Fig. 13: Average daily prey consumption by female and male *Dicyphus tamaninii* during development on 1-2- and 4-5-day-old *Aphis gossypii* individuals offered on cucumber leaves at 18±1°C. [Bars with different letters are significantly different at 5% p≤5% (Two-factor analysis of variance)]

3.1.2.2 Prey consumption by the adults

At both temperatures, *D. tamaninii* adults started feeding on *A. gossypii* soon after their emergence from the last nymphal instar. The average daily prey consumption by the females and males fluctuated irregularly over their entire longevity at 25±1°C (Fig. 14). When 1-2-day-old aphids were offered, it started with 41.3 (**&&**), 39.5 (**%%**) aphids and fluctuated hereafter to reach an average of 50.9 (**&&**), 29.9 (**%%**) on the 14th (**&&**), 11th (**%%**) day. After that the prey consumption took an irregular decreasing tendency, where it reached 18 (**&&**), 15 (**%%**) aphids on the last day of the experiments. With 4-5-day-old *A. gossypii* the prey consumption was on the 1st day 15.8 (**&&**), 15.7 (**%%**) aphids, then it increased irregularly to reach 25.4 (**&&**), 10.2 (**%%**) on the 22nd (**&&**), 23rd (**%%**) day. After that the prey consumption continued to fluctuate where it reached 13 (**&&**), 9 (**%%**) on the last experimental day.

With both prey age groups used, clear differentiation in prey consumption was noticed between both sexes starting from the 3rd day after adult emergence. When 1-2-day-old *A. gossypii* were offered, the average total prey consumption by adult *D. tamaninii* over their longevity was 844 (**&&**), 494 (**%%**), while with 4-5-day-old aphids it valued 652 (**&&**), 262 (**%%**) aphids. Statistical analysis showed that such differences in the prey consumption by adult females and males were significant with both prey age groups.

Similar tendency in the prey consumption was also found at 18±1°C, where irregular fluctuation in the prey consumption with both prey age groups was noticed (Fig. 15). When 1-2-day-old *A. gossypii* individuals were offered as prey, adult *D. tamaninii* consumed on the 1st day after their emergence 20.1 (**&&**), 17.5 (**%%**) aphids. This had increased and reached a peak of 33.8 (**&&**), 19.1 (**%%**) on the 9th (**&&**), 19th (**%%**) day. On the last day of the experiment, the last living adult consumed 6 (**&&**), 15 (**%%**) aphids. With 4-5-day-old aphids as prey, the prey consumption of the adults was 15.5 (**&&**), 15.0 (**%%**) on the 1st day and reached with 23.7 (**&&**), 15.3 (**%%**) its peak on the 11th (**&&**), 14th (**%%**) day. After that it decreased irregularly and reached 10 (**&&**), 3 (**%%**) aphids on the last day of the experiments.

Sexual differentiation in the prey consumption by the female and male *D. tamaninii* started with 1-2-day old *A. gossypii* as prey from the 6th day and with 4-5-day-old aphids from the 4th day after adult emergence. In average, *D. tamaninii* females consumed over their longevity 1611 individuals of 1-2-day-old *A. gossypii*, while the males with 700 consumed significantly less than the females. When 4-5-day-old preys were used, the average total prey consumption by the females was 783 and by the males 420, which was also significantly different.

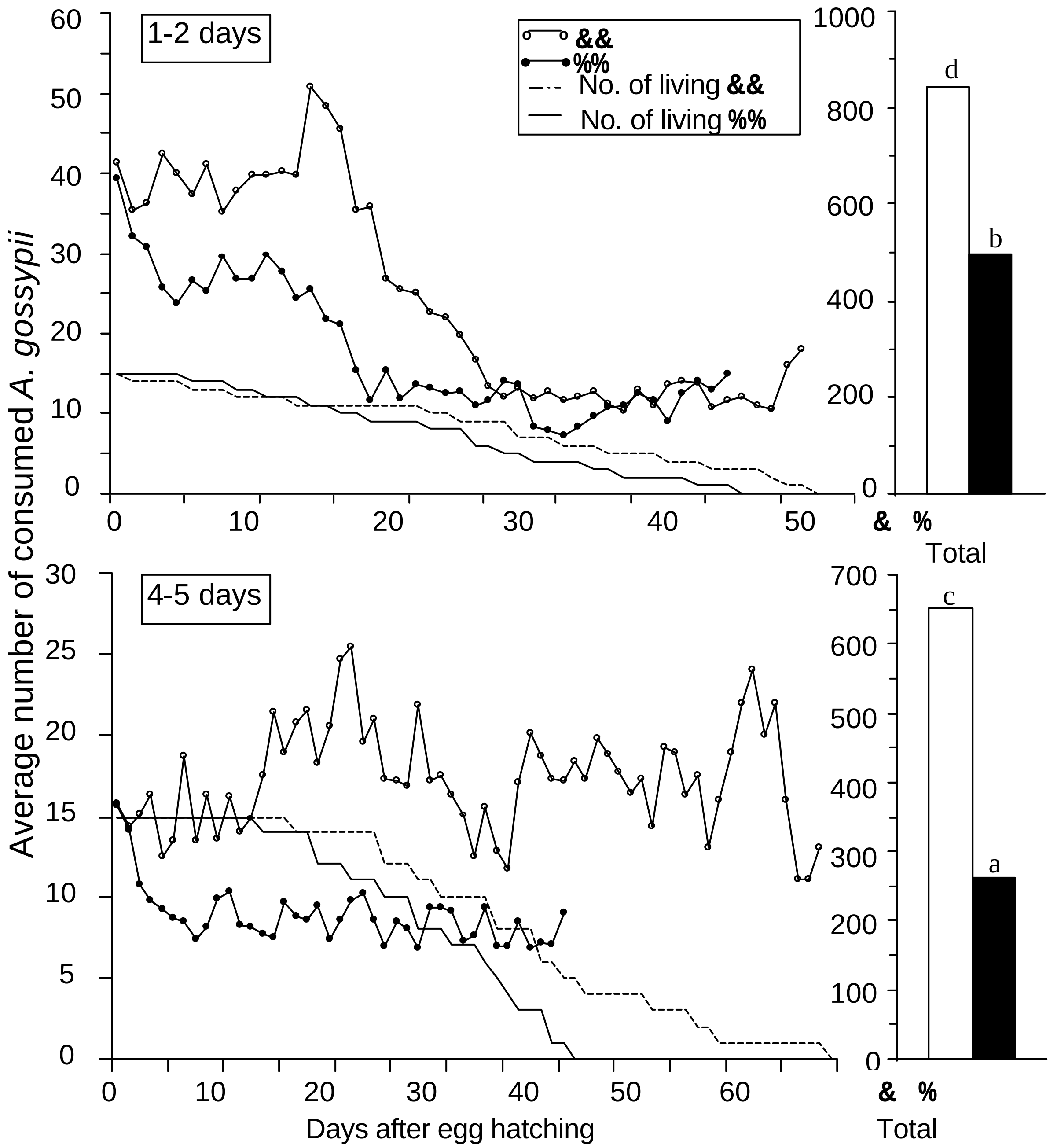

Fig. 14: Average daily prey consumption by female and male of *Dicyphus tamaninii* on 1-2- and 4-5-day-old *Aphis gossypii* individuals offered on cucumber leaves at 25±1°C. [Bars with different letters are significantly different at p≤5% (Two-factor analysis of variance)]

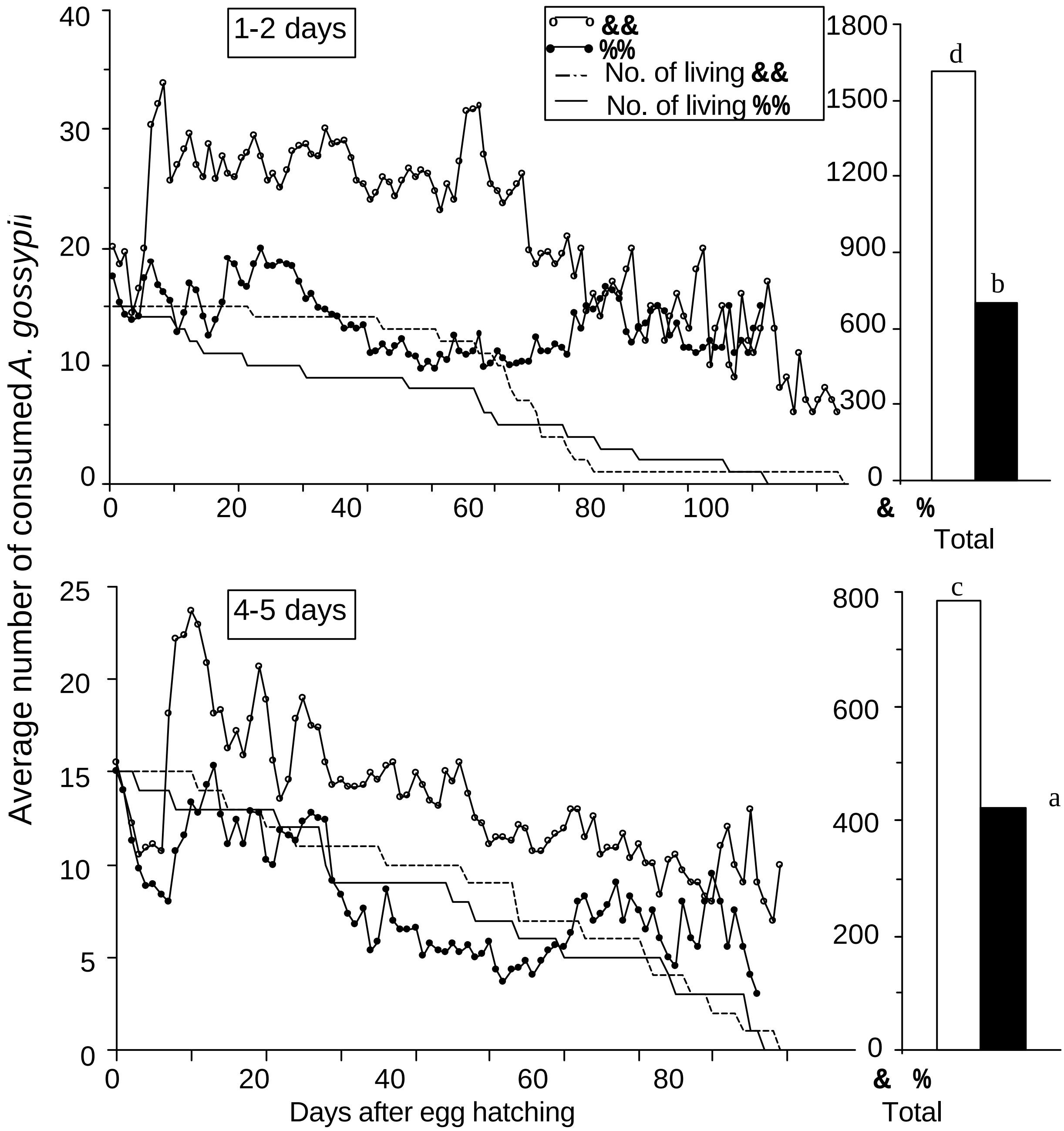

Fig. 15: Average daily prey consumption by female and male of *Dicyphus tamaninii* on 1-2- and 4-5-day-old *Aphis gossypii* individuals offered on cucumber leaves at 18±1°C. [Bars with different letters are significantly different at p≤5% (Two-factor analysis of variance)]

3.1.2.3 Prey consumption by changing prey offer

In the present set of experiments, the predatory efficiency of adult *D. tamaninii* was investigated with alternating prey offer of 1-2-day-old *A. gossypii* individuals at 25±1°C. Experiments were conducted on one-week-old *D. tamaninii* females, where 70, 15, 7, and 3 aphid/day were offered during the 1^{st} experimental week. The prey offer was changed then to 35 aphid/day during the 2^{nd} experimental week, and then was switched again to 70, 15, 7 and 3 aphid/day during the 3^{rd} week (Fig. 16).

During the 1^{st} week, when the daily prey offer was 70 aphid/day, an average of 37.8–43.9 aphid/day were consumed. While an average of 10.1-12.5 and 5.8-6.4 aphid/day were consumed when 15 and 7 aphid/day were offered, respectively. Least prey consumption of 2.5–2.9 aphid/day was recorded when 3 aphids were offered per day.

The prey consumption decreased during the second experimental week in the trial, before which 70 aphid/day had been offered. While in the other trials, before which 15, 7 and 3 aphid/day were offered, a considerable increase in the prey consumption was noticed. Such increase was clearer in the trials before which 3, 7 aphid/day were offered. During this week, one *D. tamaninii* female consumed in the 4 trials in average 17.5-34.0 aphid/day.

During the 3^{rd} experimental week, the daily prey offer was altered again to 70, 15, 7 and 3 aphid/day. In the first trial, with a daily prey offer of 70 aphids, *D. tamaninii* female consumed with 36.1-50.4 aphid/day/**&** considerably more than that during the week before. In the other trials, the prey consumption showed remarkable decrease especially in the trials, in which 7 and 3 aphid/day had been offered, where an average of 5.8-6.4 and 2.4-2.9 aphid/day/**&** were consumed, respectively.

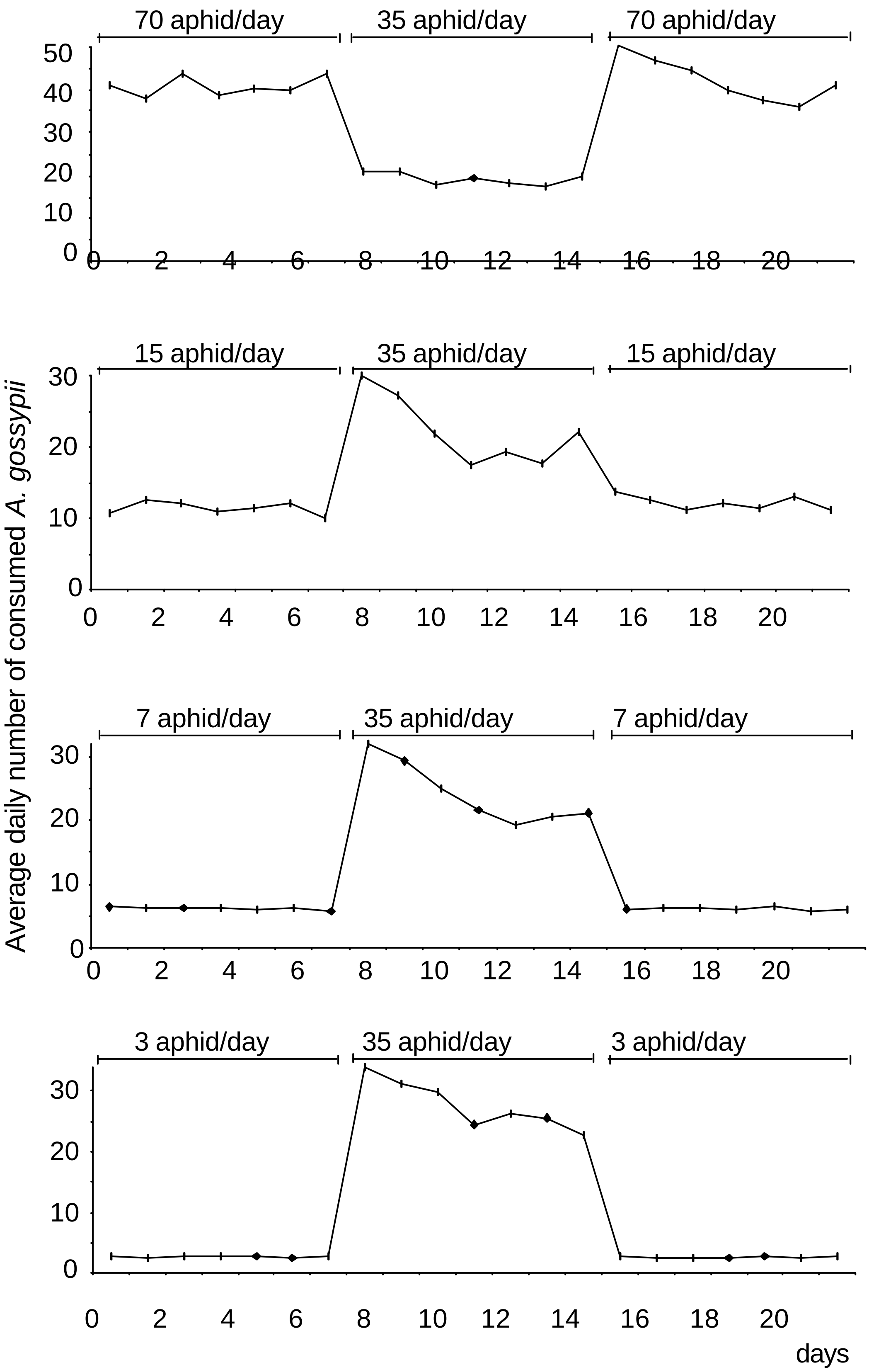

Fig. 16: Average daily prey consumption by female *Dicyphus tamaninii* with a changing number of 1-2-day-old *Aphis gossypii* individuals as prey offered on cucumber leaves at 25±1℃

3.1.2.4 Prey preference and alternative nutritional sources

3.1.2.4.1 Mixed population of *Aphis gossypii*

The prey age preference of N_3, N_5 and adult females of *D. tamaninii* was investigated with a mixed population of 1-2-, 4-5-day-old as well as adult *A. gossypii* individuals as prey.

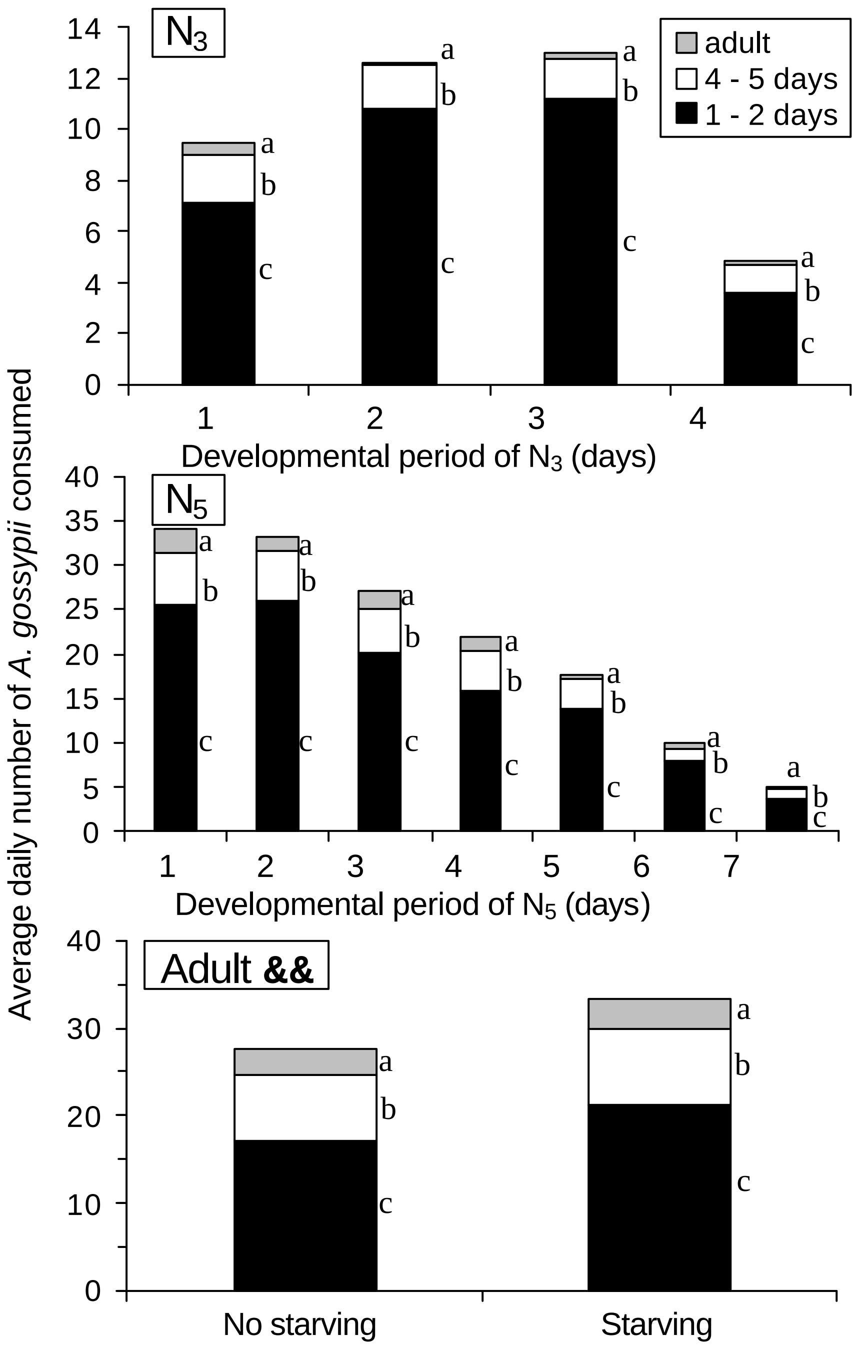

Fig. 17: Prey consumption by N_3, N_5 and one-week-old adult females of *Dicyphus tamaninii* by feeding on a mixed population of 1-2- and 4-5-day-old as well as adult *Aphis gossypii* individuals at 25±1°C. [Different letters within the same bar indicate significant differences in the prey consumption of different prey age groups at p≤5% (One-factor analysis of variance)]

The average daily numbers of *A. gossypii* from different age groups consumed by *D. tamaninii* by all tested predatory stages was significantly higher with 1-2-day-old aphids, on the other hand, adult aphids were the least preferred. As to be seen in figure 17, the third nymphal instar N_3 had consumed over its developmental period in average 7.1-11.2, 1.1-1.9 and 0.1-0.5 aphid/day from 1-2-, 4-5-day-old as well as adult *A. gossypii*, respectively. On the other hand, an average daily consumption of 3.7-25.5, 1.0-5.8 and 0.3-2.8 aphids from the three prey age groups was recorded by N_5 instar. Adult female *D. tamaninii* showed also a clear preference toward 1-2-day-old preys, where it consumed in average 17.2 aphid/day, while with 4-5-day-old as well as adult *A. gossypii* it consumed in average 7.5 and 2.9 aphid/day, respectively. This tendency in the prey age preference remained unchanged also when the predatory adult females had a starving period of 24 hours before the experiment. However, when they were starved, adult females consumed more aphids from all age groups tested, where one starved female consumed in average 20.7 (1-2), 9.0 (4-5) and 3.9 (adult) aphid/day.

3.1.2.4.2 Mixed population of different prey species

In the present experiments, the prey preference of N_3 and N_5 instars over their developmental periods as well as adult females of *D. tamaninii* was studied in a multi-choice prey offer of 10 individuals of 5 different prey species. By all stages of *D. tamaninii* tested, there was a general tendency in the prey preference toward *A. gossypii* (Fig. 18), where an average of 1.4-6.5, 2.0-8.0 and 7.3–7.5 aphid/day were consumed by N_3, N_5 and adult female, respectively. Among the other 4 prey species offered, *M. persicae* was the least preferred by all predatory stages tested, where in average 0.3–0.9 individuals were consumed by N_3, 0.3–2.3 by N_5 and 3.0–3.4 by the adult female. No clear tendency in the prey preference of *D. tamaninii* was to be distinguished among *T. vaporariorum, F. occidentalis* and *T. urticae*. It is to be mentioned also, that the prey preference toward *A. gossypii* was clearer with both nymphal stages of the predator than the adult female. Furthermore, the average daily prey consumption from all prey species had increased with development to the adult stage where it reached up to 16.1 preys by N_3, 21.3 by N_5 and 26.5 by the adult female.

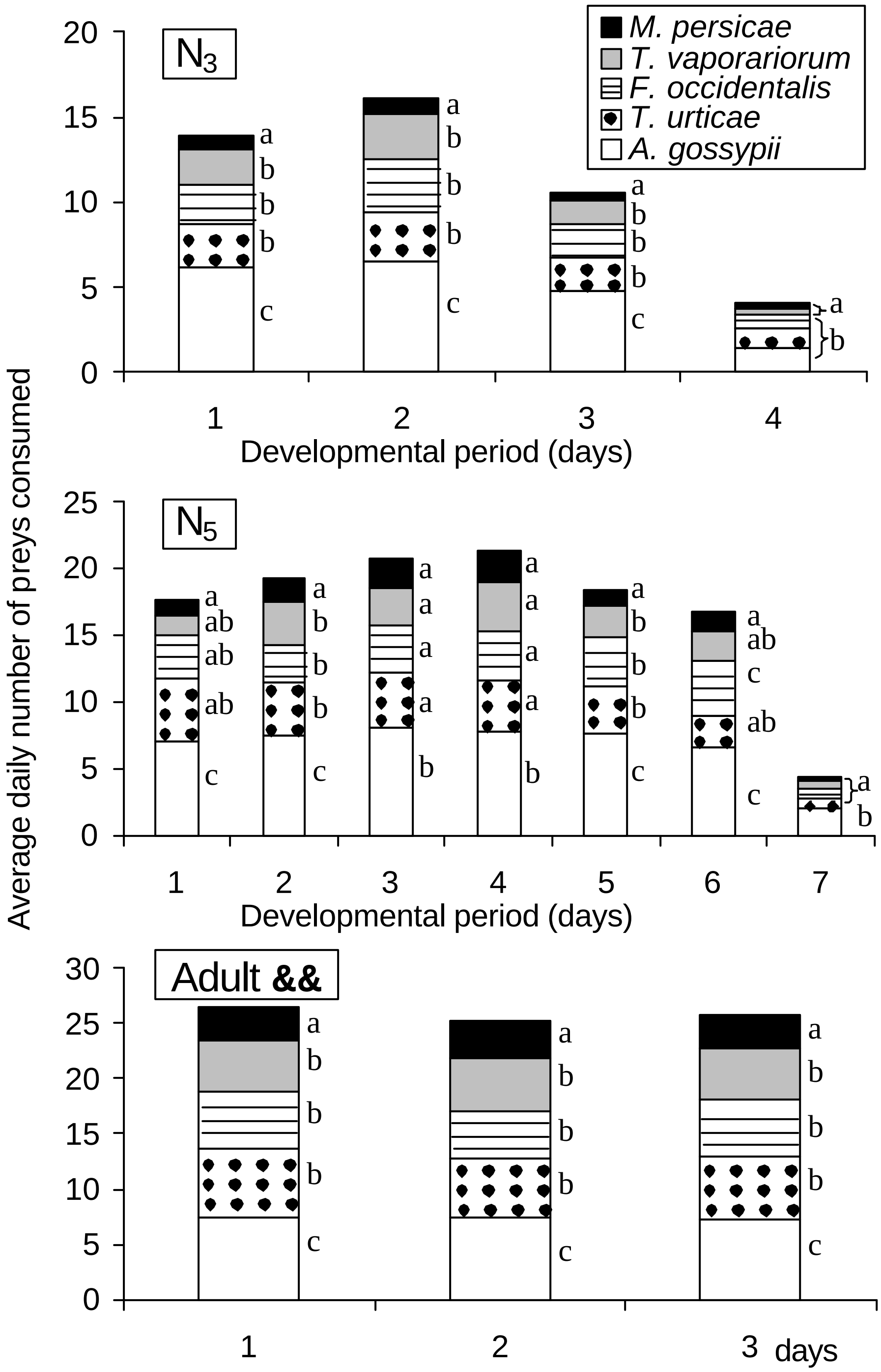

Fig. 18: Prey consumption by N_3, N_5 and adult females of *Dicyphus tamaninii* by feeding on a mixed population of 5 different prey species offered together on cucumber leaves at 25±1°C. [Different letters within the same bar indicate significant differences in the prey consumption of different prey species at p≤5% (One-factor analysis of variance)]

3.1.2.4.3 Mixed population of *Tetranychus urticae*

The red spider mite *T. urticae* is an important pest that occurs frequently on cucumber and many crops in open field as well as greenhouse. Therefore, it is very useful to investigate if *D. tamaninii* can successfully develop to adult stage when exclusively fed on *T. urticae*. The results of the experiments conducted for this purpose showed that the predatory bug was able to prey successfully on *T. urticae* and develop from N_1 to adult within a total duration of 21.2 (**&&**), 20.8 (**%%**) days (Tab. 7). Between the 5 nymphal instars, N_5 was longest where it lasted 6.8 (**&&**), 6.4 (**%%**) days. Figure 19 shows the percentage mortality of *D. tamaninii* during development on *T. urticae* as prey, where only 16 out of 20 N_1 individuals were able to reach the adult stage, i.e., 20% mortality.

Tab. 7: Average nymphal developmental period of *Dicyphus tamaninii* at 25±1°C with a mixed population of *Tetranychus urticae* as prey offered ad libitum on cucumber leaf discs

Sex	Replicates	Nymphal developmental period (days)					
		N_1	N_2	N_3	N_4	N_5	Total
&&	6	3.2	3.3	3.3	4.5	6.8	21.2±0.6 a
%%	10	3.3	3.3	3.6	4.2	6.4	20.8±0.5 a

Values with different letters are significantly different at p≤5% (T-test)

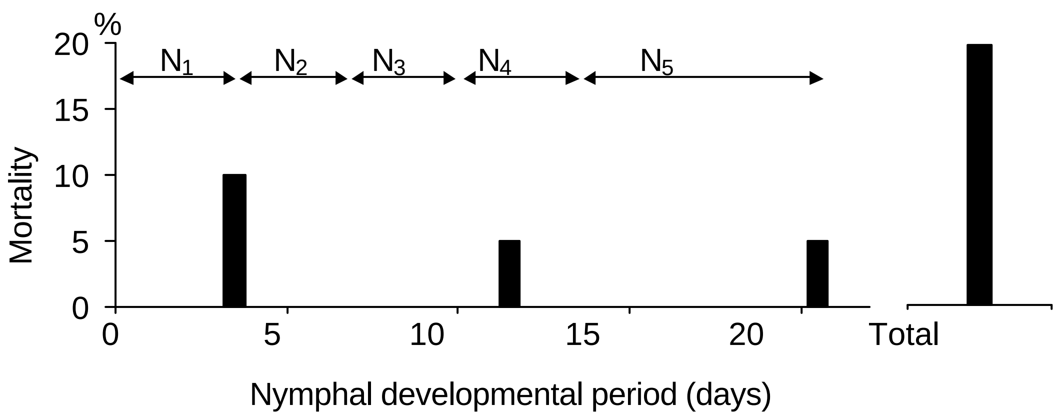

Fig. 19: Percentage mortality of *Dicyphus tamaninii* during nymphal development by feeding on a mixed population of *Tetranychus urticae* as prey offered ad libitum on cucumber leaf discs at 25±1°C

3.1.2.4.4 Alternative nutritional sources

It would be of great value for a natural enemy, if it were able to partially feed and survive on alternative nutritional sources in case of prey absence or scarcity. Figure 20 shows the results of the experiments conducted in order to determine the average survival duration of one-week-old *D. tamaninii* females when exclusively fed on different nutritional sources at 25±1°C. When *A. gossypii* was offered as prey, the predatory females lived in average 41.2 days, while with exclusively 10% honey emulsion the females lived with 14.5 days significantly shorter than that with *A. gossypii*. The survival duration was further shortened when the females were allowed to feed only on a cucumber plant, where they lived in average 9.8 days. When left without food, *D. tamaninii* females were able to survive in average for 3.8 days, which was the shortest survival duration

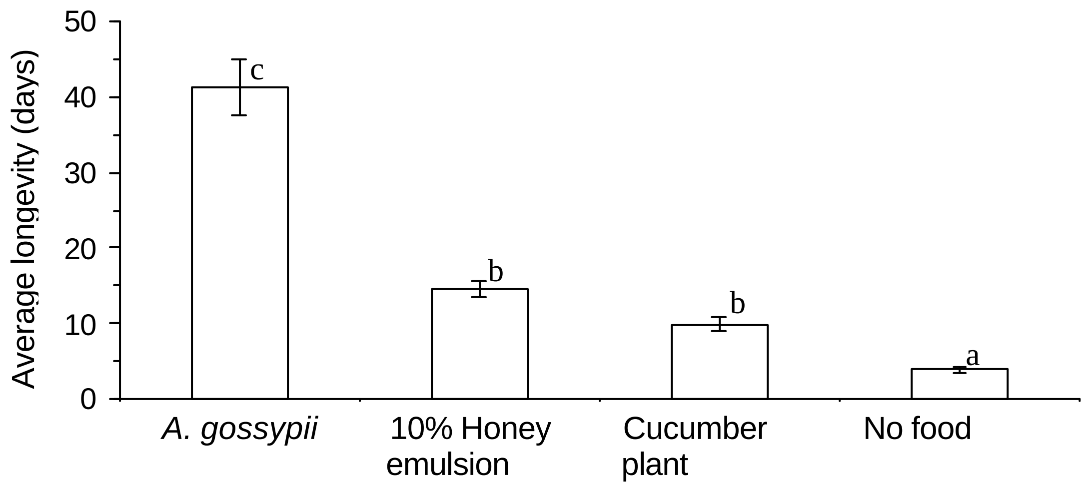

Fig. 20: Average survival duration (±S.E.) of one-week-old *Dicyphus tamaninii* females by feeding on different nutritional sources at 25±1°C. [Bars with different letters are significantly different at $p \leq 5\%$ (One-factor analysis of variance)]

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3.1.3 Effect of extreme high constant and alternating temperatures on the development, mortality and prey consumption by *Dicyphus tamaninii*

3.1.3.1 Development

The embryonic developmental period was determined at all temperatures for both sexes together. *D. tamaninii* eggs were able to develop and hatch at 30±1°C after an average of 9.6 days

(Tab. 8). While at 35±1°C, no eggs were able to hatch to nymphs, i.e., this temperature had been fatal for the eggs. *D. tamaninii* eggs were able to develop successfully at both alternating temperatures tested, where they hatched at 25/15+1°C in average after 17.7 days, significantly longer than that at 35/22±1°C, where they hatched after 10.8 days (Tab. 9).

Tab. 8: Average embryonic developmental period of *Dicyphus tamaninii* at two different high constant temperatures

Temperature °C	Replicates	Embryonic developmental period (days)	
		Average ± SE	Min. – Max.
30±1	24	9.6±0.1	9 - 10
35±1	25	No hatching	

Tab. 9: Average embryonic developmental period of *Dicyphus tamaninii* at two different alternating temperatures

Temperature °C	Replicates	Embryonic developmental period ± SE (days)	
		Average ± SE	Min. – Max.
25/15±1	22	17.7±0.2 a	16 -19
35/22±1	19	10.8±0.3 b	10 - 12

Values with different letters are significantly different at p≤5% (T-test)

The experiments on the nymphal development of *D. tamaninii* were conducted on 15 nymphs at each constant temperature of 30±1°C and 35±1°C (Tab. 10). The predator was able to develop successfully and reach the adult stage in average after 20.1 (**&&**), 20.6 (**%%**) days. On the other hand, it was not able to develop to the adult stage at 35±1°C, where all N_1 instars used at the beginning of the experiments had died even before moulting to N_2 instar. Table 11 shows the average nymphal developmental periods of 25 and 24 *D. tamaninii* individuals tested at the alternating temperatures of 25/15±1°C and 35/22±1°C, respectively. At 25/15±1°C, the predator reached the adult stage 23.7(**&&**), 23.5 (**%%**) days after egg hatching, while when the temperature was increased to 35/22±1°C it took with 20.6 (**&&**), 20.9 (**%%**) days clearly shorter period. At all constant and alternating temperatures, no significant differences were found in the average nymphal developmental periods of the female and male *D. tamaninii*.

Tab. 10: Average nymphal developmental period of *Dicyphus tamaninii* at two different high constant temperatures with 1-2-day-old *Aphis gossypii* as prey offered ad libitum on cucumber leaf discs

Temperature °C	Replicates	Sex	Nymphal developmental period (days)				
			N_1	N_2	N_3	N_4	N_5
30±1	7	**&&**	2.3	3.3	4.0	4.1	6.4
	8	**%%**	2.5	3.2	4.2	4.3	6.4
35±1	15		No further development				

Tab. 11: Average nymphal developmental period of *Dicyphus tamaninii* at two different alternating temperatures with 1-2-day-old *Aphis gossypii* as prey offered ad libitum on cucumber leaf discs

Temperature °C	Replicates	Sex	Nymphal developmental period (days)				
			N_1	N_2	N_3	N_4	N_5
25/15±1	13	**&&**	4.7	3.4	4.2	4.0	7.5
	12	**%%**	4.8	3.2	4.1	4.1	7.4
35/22±1	11	**&&**	3.4	3.5	3.3	3.5	7.0
	13	**%%**	3.6	3.6	3.2	3.6	6.8

Figure 21 represents the average total developmental period of *D. tamaninii* from the egg till the adult stage with 1-2-day-old *A. gossypii* individuals as prey at different constant and alternating temperatures. At the constant temperature of 30±1°C, the predatory bug developed from the egg till the adult stage in an average of 29.7 (**&&**), 30.2 (**%%**) days. On the other hand, at 35±1°C the predator was not able to successfully develop neither during the egg stage nor the nymphal stage. At the alternating temperature of 25/15±1°C, the total developmental period was in average 41.4 (**&&**), 41.2 (**%%**) days, while at 35/22±1°C it was 31.4 (**&&**), 31.7 (**%%**) days.

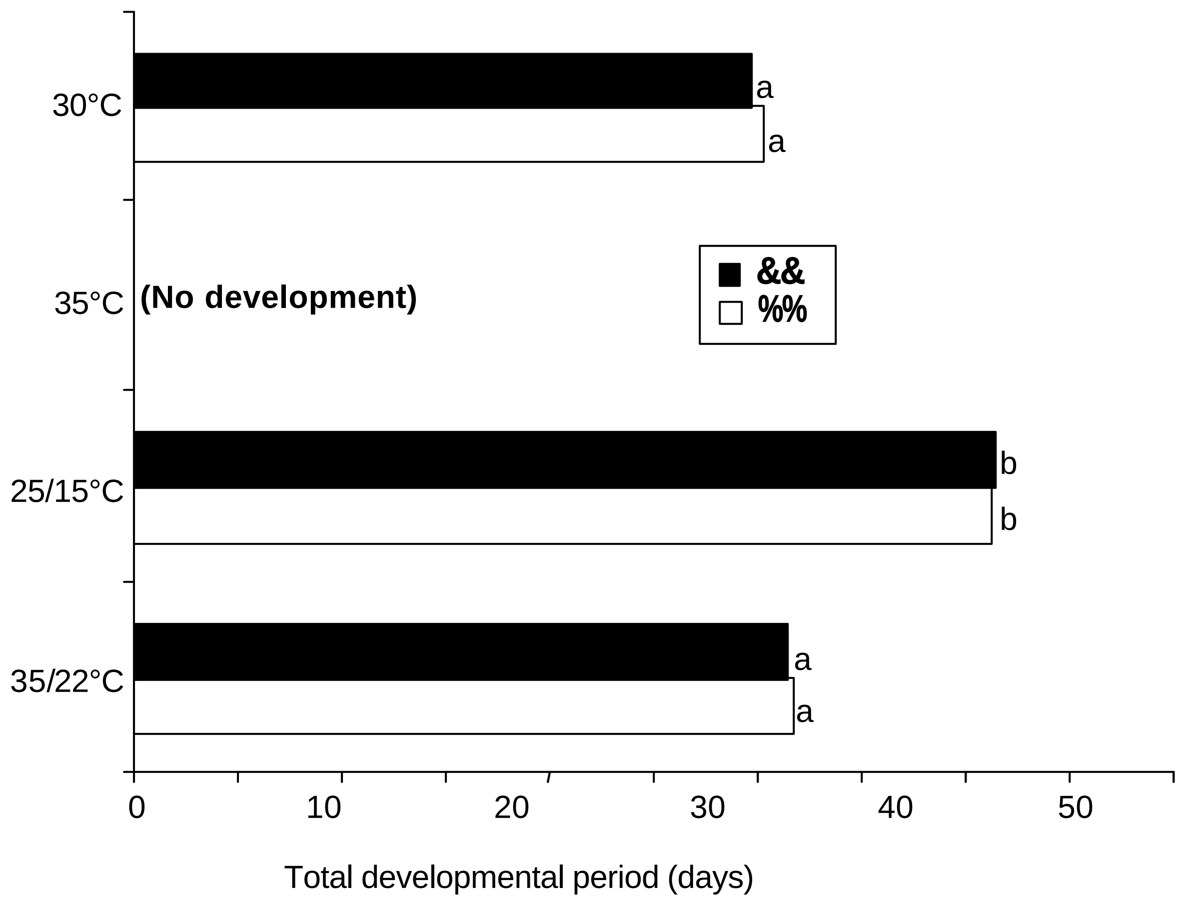

Fig. 21: Average total developmental period from the egg till the adult stage of *Dicyphus tamaninii* at different constant and alternating temperatures with 1-2-day-old *Aphis gossypii* as prey. [Bars with different letters are significantly different at $p \leq 5\%$ (Two-factor analysis of variance)]

3.1.3.2 Mortality

The percentage mortality of *D. tamaninii* during development at two high constant and two alternating temperatures with 1-2-day-old *A. gossypii* as prey is represented in figure 22. In general, highest mortality occurred during the first two nymphal instars. At the constant temperature of 30±1°C, the total percentage mortality during development from N_1 to adult stage was 30%. A total percentage mortality of 100% occurred at 35±1°C, where all N_1 nymphs used at the beginning of the experiments died before even moulting to N_2 instar. At the alternating temperature of 25/15±1°C, the total percentage mortality was with 16.5% the lowest, while at 35/22±1°C it rose slightly and valued 20%.

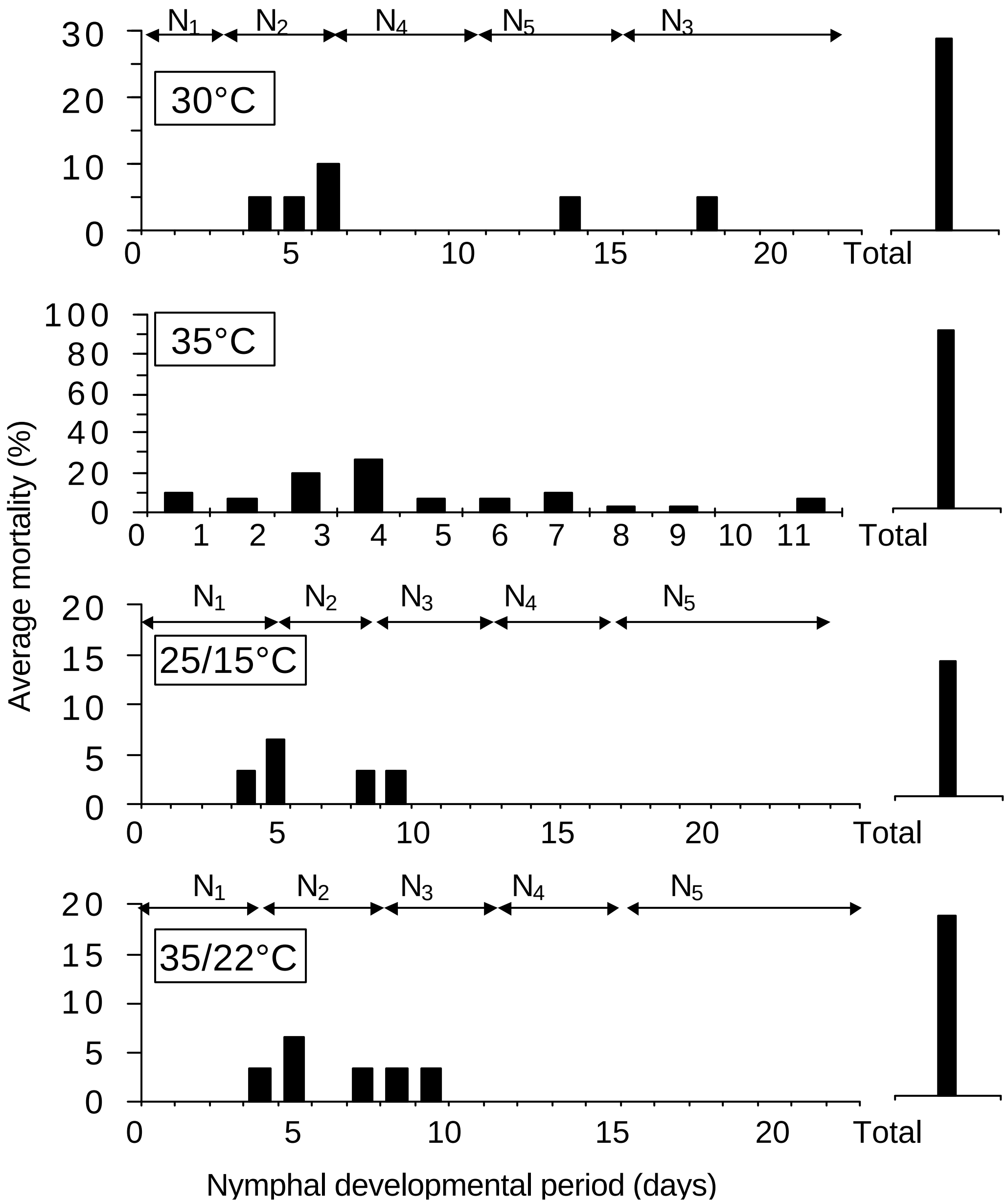

Fig. 22: Percentage mortality of *Dicyphus tamaninii* during nymphal development by feeding on 1-2-day-old *Aphis gossypii* on cucumber leaves at different high constant and alternating temperatures

3.1.3.3 Prey consumption

3.1.3.3.1 Effect of high temperature on the prey consumption by the nymphal instars

Experiments on the effect of two extreme high constant and alternating temperatures on the prey consumption by *D. tamaninii* during its development were conducted using 1-2-day-old *A. gossypii* individuals as prey. At the constant temperature of 30±1°C (Fig. 23), one *D. tamaninii* nymph consumed on the 1^{st} day after egg hatching in average 3.7 (**&&**), 5.8 (**%%**) aphid/day. The prey consumption increased gradually with development and reached its peak of 49.0 (**&&**), 44.0 (**%%**) aphid/day on the 15^{th} (**&&**), 18^{th} (**%%**) day. In total, an individual of *D. tamaninii* consumed during its development from N_1 to adults at 30±1°C an average of 442.2 (**&&**), 433.6 (**%%**) aphid/day. At the constant temperature of 35±1°C, the average daily prey consumption started with 4 aphids and decreased continuously till it approached nil on the 9^{th} day, where all experimental predatory nymphs died.

At the alternating temperature of 25/15±1°C, the average daily prey consumption was on the 1^{st} day 3.1 (**&&**), 3.3 (**%%**) aphids and reached a peak of 35.3 (**&&**), 24.1 (**%%**) aphids on the 20^{th} day. In total, during development from N_1 to adult, one *D. tamaninii* individual consumed in average 359.3 (**&&**), 297.0 (**%%**) aphids. The average daily prey consumption by *D. tamaninii* at 35/22±1°C increased gradually with development, where it was 2.1 (**&&**), 2.7 (**%%**) aphids on the 1^{st} day and reached up to 46.9 (**&&**), 32.3 (**%%**) aphids on the 20^{th} (**&&**), 18^{th} (**%%**) day. Until it reached the adult stage, one *D. tamaninii* nymph consumed in average a total of 348.5 (**&&**), 334.0 (**%%**) aphids. As to be seen in figure 23, *D. tamaninii* nymphs consumed during their development significantly more *A. gossypii* at 30±1°C than at other temperatures. On the other hand, except that at 25/15±1°C, the average total prey consumption by female and male *D. tamaninii* showed no significant differences.

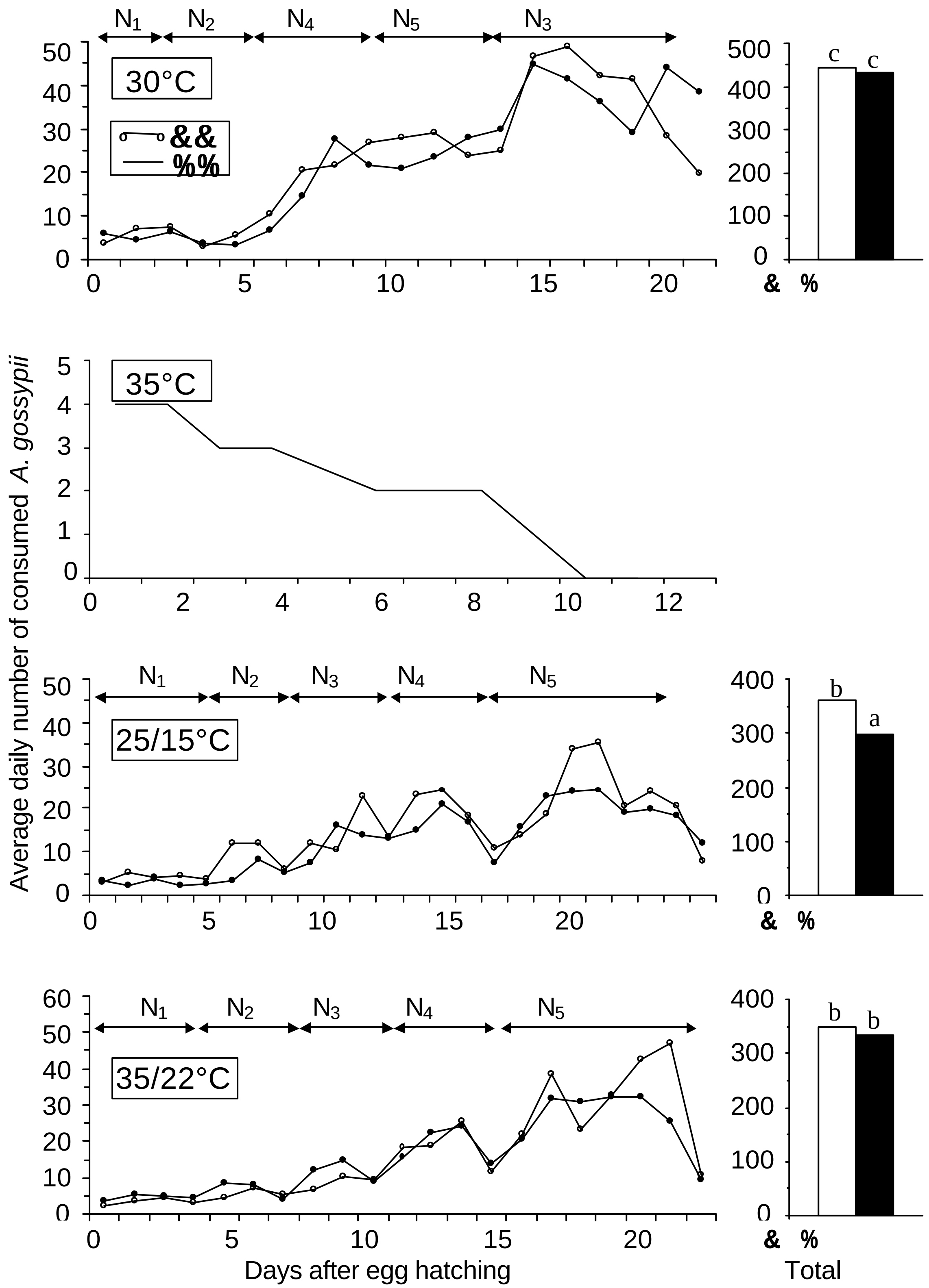

Fig. 23: Average daily prey consumption by female and male *Dicyphus tamaninii* during development on 1-2-day-old *Aphis gossypii* individuals as prey offered on cucumber leaves at different constant and alternating temperatures. [Bars with different letters are significantly different at p≤5% (Two-factor analysis of variance)]

3.1.3.3.2 Effect of high temperature on the prey consumption by the adults

The prey consumption by 20 adult *D. tamaninii* was investigated with *A. gossypii* individuals as prey at each of the high constant temperatures of 30±1°C and 35±1°C as well as the alternating temperatures of 25/15±1°C and 35/22±1°C (Fig. 24)

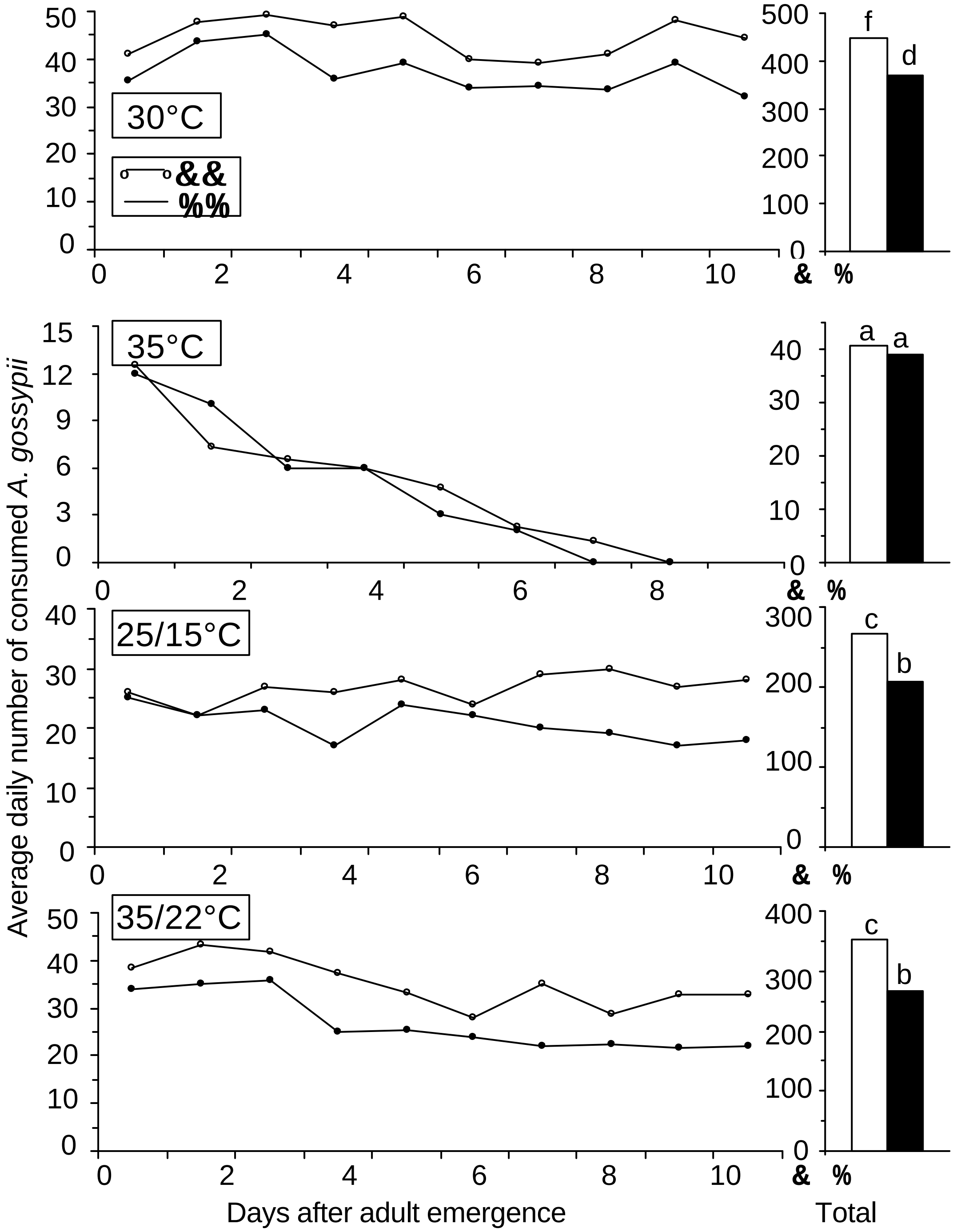

Fig. 24: Average daily prey consumption by female and male *Dicyphus tamaninii* when fed on 1-2-day-old *Aphis gossypii* individuals as prey offered on cucumber leaves at different high constant and alternating temperatures. [Bars with different letters are significantly different at p≤5% (Two-factor analysis of variance)]

At all temperatures tested, adults started feeding soon after emerging from the last nymphal instars. The daily prey consumption by adult *D. tamaninii* at 30±1°C showed a slight fluctuation and ranged in average from 39.0–49.2 (**&&**), 32.0–45.0 (**%%**) aphids per day. In average, one *D. tamaninii* adult consumed during the first 10 days after emergence a total of 446.4 (**&&**), 372.0 (**%%**) aphids. As it was during development, the temperature of 35±1°C was also fatal to adult *D. tamaninii*, where the average daily prey consumption was with 12.5 (**&&**), 12.0 (**%%**) aphids highest on the 1^{st} day, then it decreased continuously to approach nil on the 8^{th} day, on which all experimental adults had been dead, after having consumed an average total of 40.6 (**&&**), 39.0 (**%%**) aphids.

The average daily prey consumption at the alternating temperature 25/15±1°C ranged from 22.0–30.0 (**&&**), 17.0–25.0 (**%%**) aphids, by the end of the 10^{th} day, one *D. tamaninii* adult will have consumed in average a total of 267.0 (**&&**), 207.0 (**%%**) aphids. Increasing the temperature to 35/22±1°C resulted in a higher average daily prey consumption by the adult predator, where it fluctuated from 27.9–43.2 (**&&**), 21.8–36.0 (**%%**) with a total of 351.5 (**&&**), 267.7 (**%%**) aphids.

3.1.4 Host plant spectrum for oviposition by *Dicyphus tamaninii*

In the present study, six different potted crop plants (tomato, tobacco, beans, sweet pepper, cucumber and eggplant) were offered simultaneously as host plants for oviposition. Table 12 represents the daily number of eggs laid by 15 *D. tamaninii* females on different plant species on the period from the 8^{th} to 14^{th} day of longevity. Results showed that *D. tamaninii* was able to lay eggs on all plant species tested. However, it was clear that among the six plant species used, cucumber with a total of 51 eggs and tobacco with 47 eggs had significantly the highest counts of *D. tamaninii* eggs. These were followed by tomato with 44 eggs, which was also significantly higher than beans, sweet pepper, and eggplant, which had 21, 17 and 14 eggs respectively.

Tab. 12: Average daily and total number of eggs laid by 15 *Dicyphus tamaninii* females from the 8^{th} till the 14^{th} days of longevity by feeding on *Aphis gossypii* as prey on different host plants

Host plant	Number of laid eggs on the							
	8^{th}	9^{th}	10^{th}	11^{th}	12^{th}	13^{th}	14^{th} day	Total
Cucumber	5	12	9	9	6	4	6	51±1.1 e
Tobacco	4	9	3	14	8	7	2	47±1.6 d
Tomato	0	4	6	16	10	5	3	44±2.0 c
Beans	0	8	7	1	0	1	4	21±1.3 b
Sweet pepper	0	4	4	3	1	1	4	17±0.6 ab
Egg plant	0	1	1	4	2	1	5	14±0.7 a

Values with different letters are significantly different at $p \leq 5\%$ (One-factor analysis of variance)

3.1.5 Interaction of *Dicyphus tamaninii* with selected beneficial arthropods

In nature as well as under the conditions of managed agro-ecosystems, several natural enemies co-exist usually together in the same habitat. As *D. tamaninii* is a polyphagous predator that might possibly prey on other available beneficial arthropods, it is worthy to investigate its interaction with other natural enemies that are commonly released in greenhouses and might serve as potential prey for the predatory bug. Therefore, the predatory behaviour of *D. tamaninii* females was studied when individuals of *A. cucumeris*, *P. persimilis* and *A. colemani* inside *A. gossypii* mummies as well as unparasitized *A. gossypii* nymphs were confined together with one predatory female for 24 hours. As to be seen in figure 25, one *D. tamaninii* female consumed in average 8.8 *A. gossypii*, which was significantly more than all other species. Although the predatory bug consumed with an average of 3.8 more *A. cucumeris* than that of *P. persimilis*, from which in average 2.6 individuals were killed. Statistical analysis showed that such difference was not significant. Least preferred as prey was *A. gossypii* mummies containing the endoparasitioid *A. colemani*, from which only an average of 0.2 individuals were attacked.

Furthermore, a 2-hour-long observation under binocular revealed that more than 70% of the consumed *A. gossypii* had been attacked within this period after launching the experiment. During the first 2 hours, almost no predatory mites or *A. gossypii* mummies were attacked by *D. tamaninii*. The interaction between the two predatory mites was also observed, where no intraguild predation between both species was recorded.

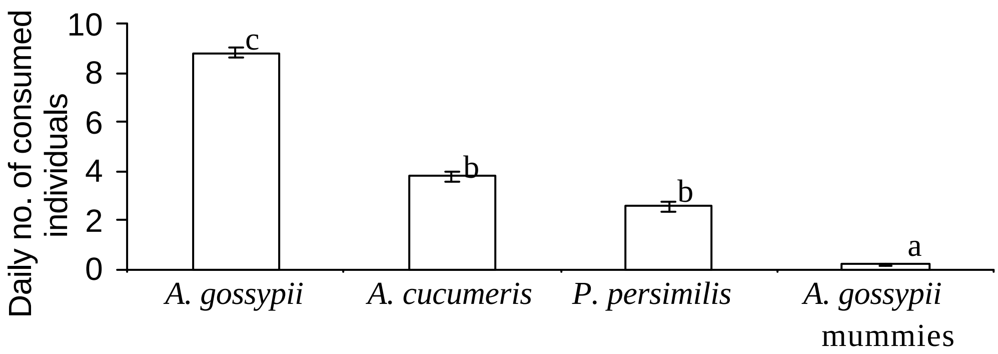

Fig. 25: Average daily number of consumed aphids, predatory mites or mummies containing *Aphidius colemani* larvae when confined together with one-week-old adult *Dicyphus tamaninii* females at 25±1°C. [Bars with different letters are significantly different at p≤5% (One-factor analysis of variance)]

3.1.6 Potential damage to cucumber fruits caused by *Dicyphus tamaninii* adults

Plant-feeding habit had been observed with *D. tamaninii* during several experiments in the present study, where feeding punctures had been frequently encountered on cucumber leaves used for the experiments. Therefore, it was necessary to investigate to what extent *D. tamaninii* could be harmful to cucumber and also if the leaves or fruits would be preferred by the predator under different nutritional conditions.

3.1.6.1 Damage to fruits with different prey treatments

Two experiments were set up in order to investigate the potential damage *D. tamaninii* can cause to cucumber fruits. The first one aimed to study how the presence or absence of leaves and prey during the experiment will affect the fruit feeding by the predator. The second experiment, on the other hand, was designed to investigate how the nutritional status of the predatory females prior to the experiment will affect their feeding on fruits.

Figure 26 represents the average number of feeding punctures on one cucumber fruit caused by 5 *D. tamaninii* females when confined together for 5 days with different prey treatments. The number of feeding punctures on the fruits was significantly affected by the presence of the leaf as well as of the prey. Substantial feeding on cucumber fruits by *D. tamaninii* occurred particularly when the cucumber leaves were not provided, where an average of 52.7 punctures was recorded. When a cucumber leaf without *A. gossypii* was provided with the fruit, the average number of feeding punctures on one fruit was significantly reduced to 36.2 punctures. Providing 25 *A. gossypii*/leaf/day had resulted in a significant reduction in the fruit feeding by the predator, where an average of 21.0 was recorded. Least fruit feeding activity of 13.2 punctures was found when excess number of *A. gossypii* was offered. When *E. kuehniella* eggs were offered ad libitum, the number of feeding punctures per fruit was increased again to reach in average 21.0 punctures.

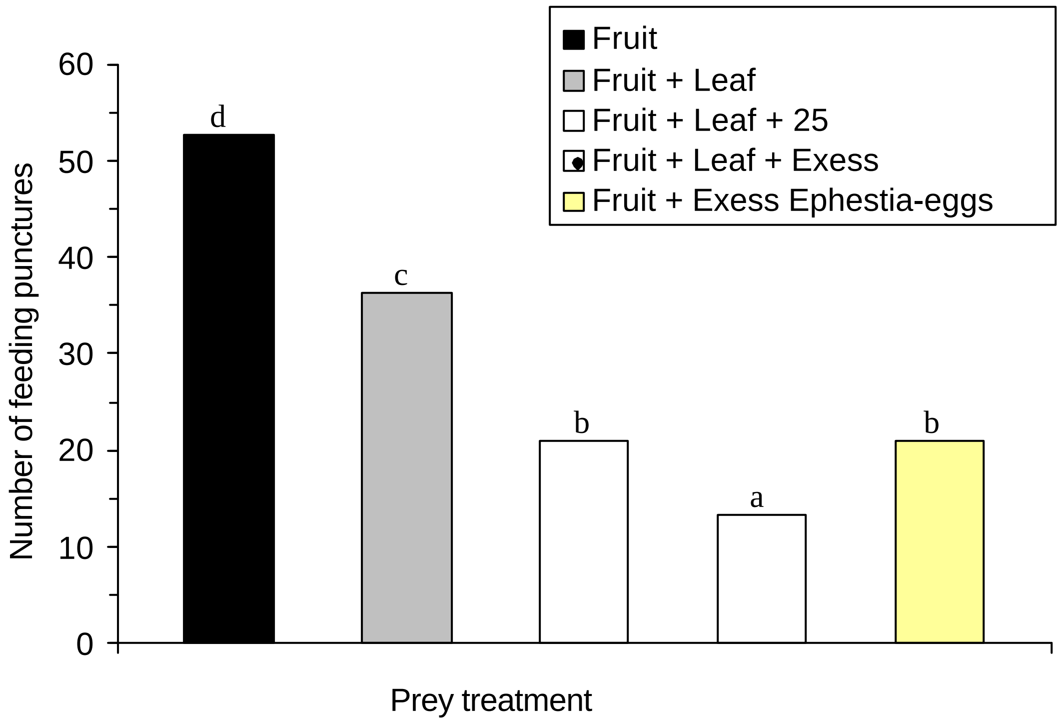

Fig. 26: Average number of feeding punctures made by 5 adult *Dicyphus tamaninii* females on cucumber fruit in the presence or absence of cucumber leaf, with different prey treatments for 5 days at 25±1°C. [(Bars with different letters are significantly different at p≤5% (One-factor analysis of variance)]

Figure 27 shows the average daily number of feeding punctures caused by *D. tamaninii* when they were offered only cucumber fruits and had been allowed to feed only on *A. gossypii*, cucumber plant or starved for 24 hours before the experiment. When the predatory females had been fed on *A. gossypii* prior to the experiment, only an average of 2.2 puncture/day/**&** was recorded. While by feeding on a cucumber plant only, the number was significantly increased to 3.1 puncture/day/**&**. Highest number of feeding punctures was encountered when the females had been starved 24 hours prior to the experiment, where one *D. tamaninii* female made in average 4.7 puncture/day.

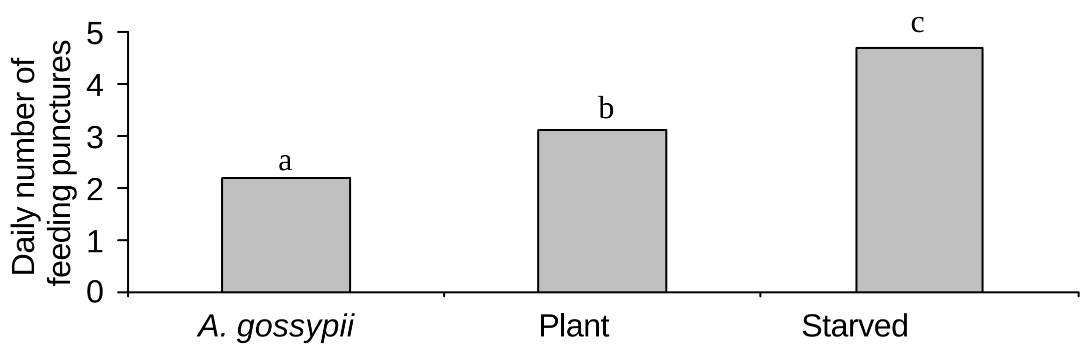

Fig. 27: Average daily number of feeding punctures caused by one adult *Dicyphus tamaninii* female confined to a cucumber fruit after being fed only on *Aphis gossypii*, cucumber plant or were starved for 24 hours before the experiments at 25±1°C. [Bars with different letters are significantly different at p≤5% (One-factor analysis of variance)]

3.1.6.2 Leaf-fruit preference

In order to investigate if the leaf or the fruit of cucumber would be preferred by *D. tamaninii*, experiments were set up in which the probability that the predatory females would be found on leaf, fruit or elsewhere in the experimental cage was quantified. Twenty-four hours prior to the

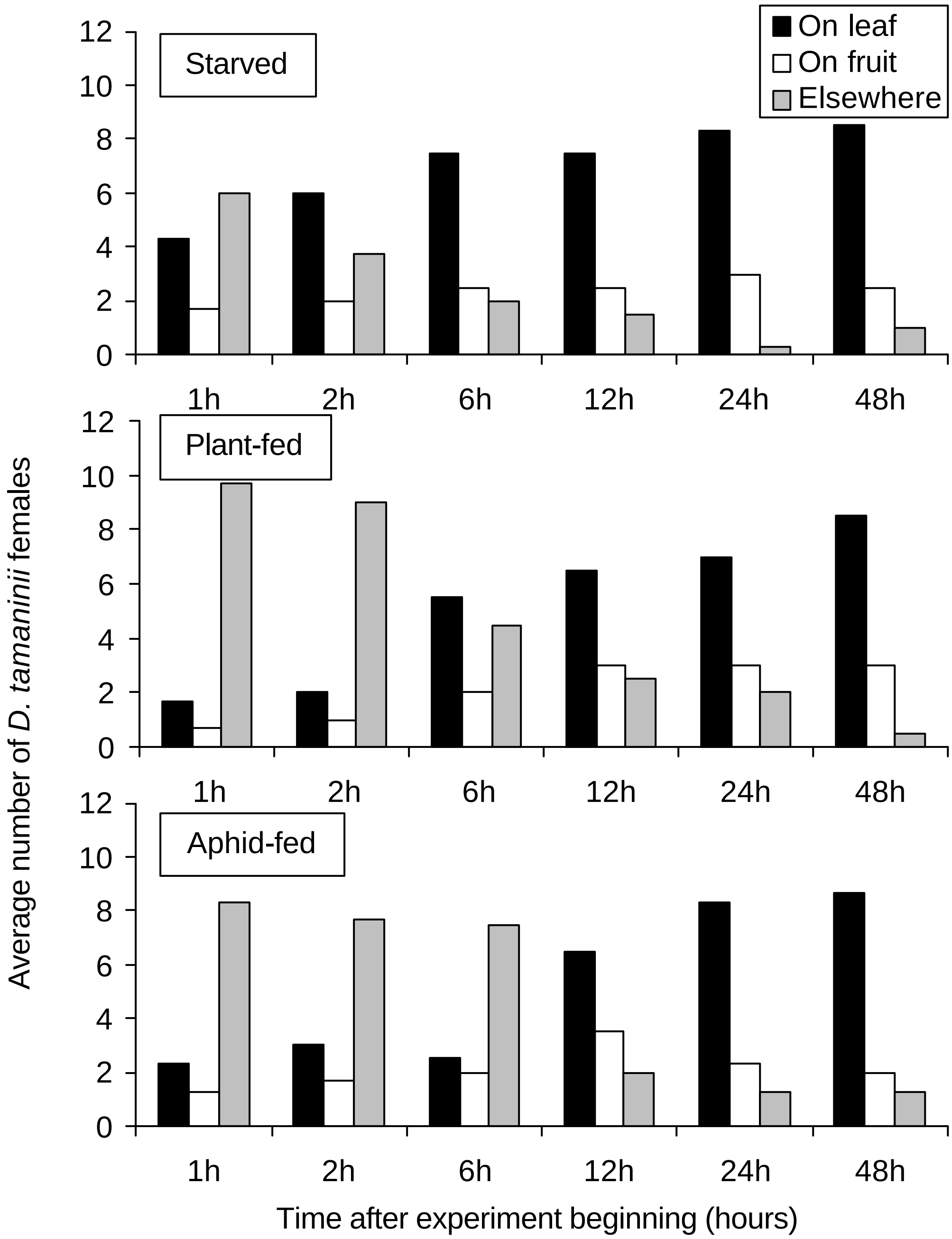

Fig. 28: Average number of *Dicyphus tamaninii* females encountered on cucumber leaf, fruit or elsewhere in the experimental cage at certain time intervals, when the females had been treated with three prey treatments 24 hours before the experiments at 20$\pm$3°C

experiments, *D. tamaninii* females were either “aphid-fed”, “plant-fed” or “starved”. As figure 28 shows, the probability that the predatory females would be encountered on leaf or fruit was generally higher than elsewhere in the cage.
Substantial increase in the probability that the predator would be found on leaf or fruit was only noticed after 12 hours with the “aphid-fed” predators, 6 hours with the “plant-fed” and only after 1 hour with the “starved” ones, where they started immediately seeking out the leaves, and were more frequently encountered on leaves or fruits. Regardless the nutritional source offered prior to the experiments, all *D. tamaninii* females were generally observed more often on cucumber leaves than on fruits.

3.2 Greenhouse experiments

Results revealed that by early release of *D. tamaninii* in cabin I (Fig. 29), the total number of *A. gossypii* from different developmental stages was in the 1st week after infestation 138 aphid/plant, this had continuously increased till it reached a maximum of 221 aphid/plant in the 4th week. The number of *A. gossypii* individuals per plant had hereafter decreased continuously where it reached an average of 59 in the last experimental week. On the other hand, the number of *A. gossypii* in cabin II was 106 aphid/plant in the 1st week and increased to reach a maximum of 364 aphid/plant in the 4th week after infestation, then it had decreased gradually till it reached an average of 98 aphid/plant in the last week of the experiment. The number of *A. gossypii* of different developmental stages was significantly higher in cabin II than that in cabin I. The number of aphids in the control cabin (III) was in general significantly higher than both other cabins, where it increased continuously from an average of 109 in the 1st week after infestation to 998 aphid/plant in the 4th week. It had taken hereafter a declining tendency where it reached in average 613 aphid/plant in the last experimental week.

Results showed that *D. tamaninii* was able to successfully feed, reproduce and establish on cucumber under greenhouse conditions with *A. gossypii* as prey. The number of the different developmental stages of the predator increased gradually with time where it was in cabin I in average 2 adults, 17 nymphs and 20 eggs per plant in the 4th week after infestation with the aphid, and increased to reach 19, 17 and 121 adults, nymphs and eggs per plant, in the last experimental week, respectively. In cabin II, the number of *D. tamaninii* was in the 4th week 2 adults, 6 nymphs and 30 eggs per plant. It took hereafter an increasing tendency where it valued per plant in average 19 adults, 24 nymphs and 63 eggs in the last week of the experiment.

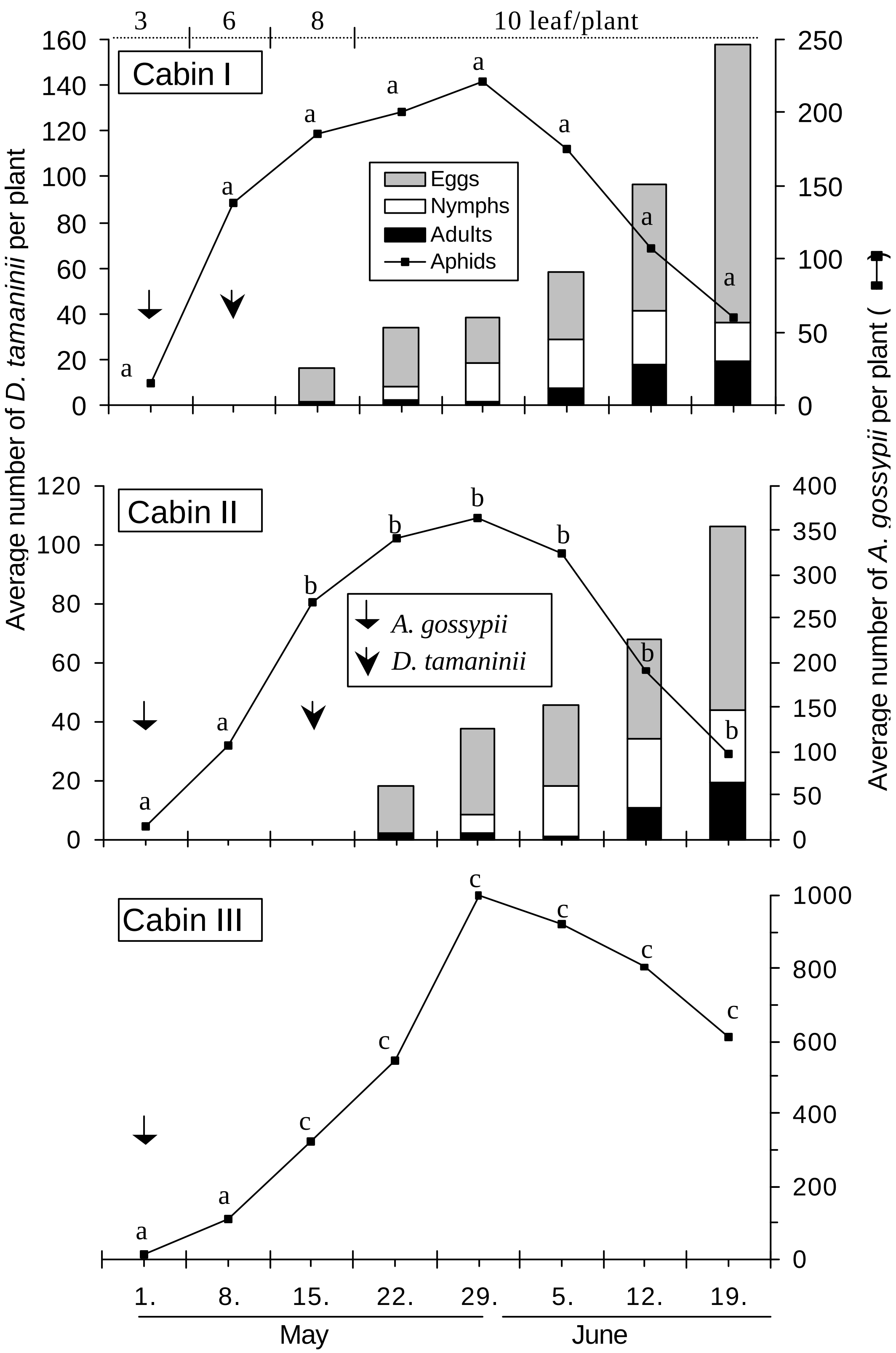

Fig. 29: Average weekly numbers of *Aphis gossypii* (all stages), and *Dicyphus tamaninii* (adult, nymph and egg stages) per cucumber plant when the predator was released 1 week (cabin I) and 2 weeks (cabin II) after infestation with the aphid, cabin III is control treatment. [In each week, numbers of *Aphis gossypii* in the three cabins with different letters are significantly different at p≤5% (One-factor analysis of variance)]

Figure 30 shows the percentage reduction in *A. gossypii* population in both cabin I and II, compared to that in the control cabin (III). In cabin I, the aphid population was reduced by 42% one week after *D. tamaninii* release, the reduction in the aphid population increased generally with time where it was 78% in the 3rd week and reached to 87% in the 5th week. Maximum reduction of 90% in *A. gossypii* population was achieved in cabin I in the 6th week. In cabin II, where the predator was released 2 weeks after infestation with the melon aphid, the percentage reduction in *A. gossypii* population was 38% in the 1st week after the release of the predator, and increased to 65% in the 3rd week, which was clearly less than that in cabin I in the same week. The reduction in *A. gossypii* population in cabin II reached its maximum in the 5th week, where it was 84%.

Behavioural observations on the movement and location of the adult *D. tamaninii* among the cucumber plants within the experimental cabins revealed that the predatory adult individuals were usually more concentrated on the plants with high prey density.

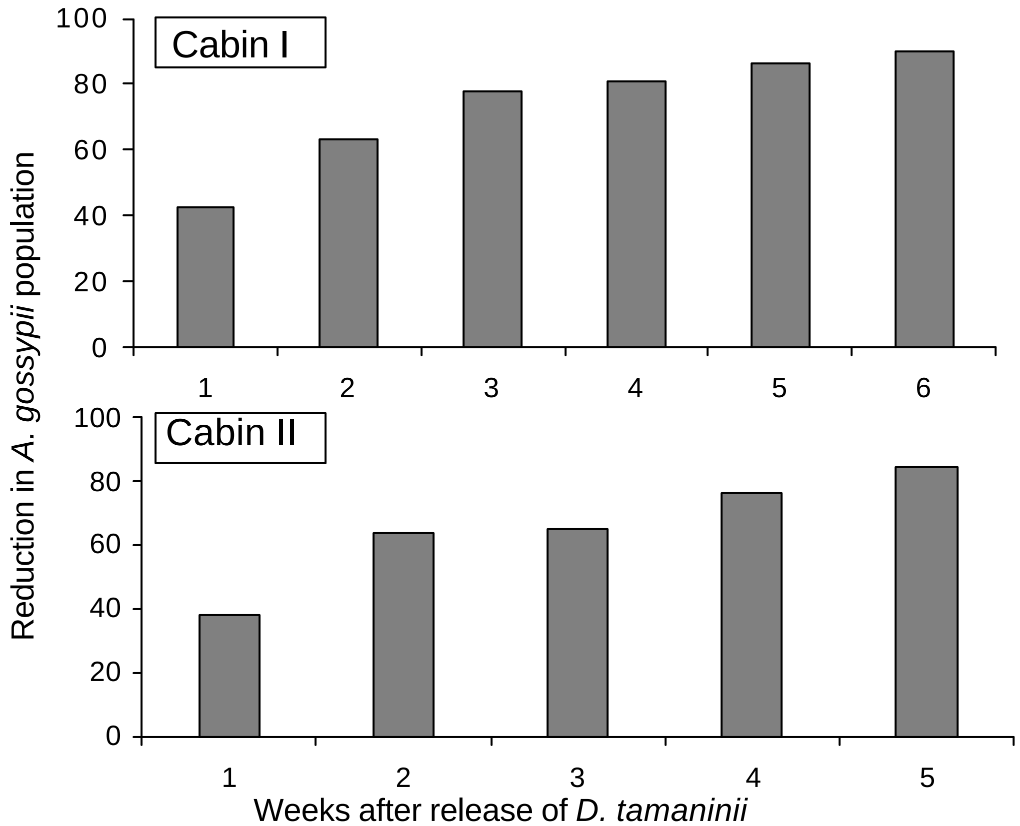

Fig. 30: Percentage reduction in *Aphis gossypii* population on cucumber compared with the control treatment when *Dicyphus tamaninii* was released 1 week (cabin I) and 2 weeks (cabin II) after aphid infestation

4 DISCUSSION

Biology

The results of the experiments showed that *D. tamaninii* was able to successfully feed and complete its development at the temperatures 25±1°C and 18±1°C when fed on 1-2- or 4-5 day-old individuals of *A. gossypii*. Furthermore, when fed on the same prey age, there were no significant differences in the developmental periods between females and males. CASTANE et al. (1997) reported that *D. tamaninii* completed its nymphal development when fed on young individuals of *A. gossypii* at 25±1°C, but no records were available at other temperatures or prey age groups. ALVARADO et al. (1997) studied the efficiency of the same predatory bug on two aphid species and found that, by feeding on *A. gossypii*, it completed its development from egg hatch to adult in 21.5 days, which agrees with what was found when 1-2-day-old *A. gossypii* were used as a prey in the present study. Furthermore, according to ALBAJES et al. (1996) the total developmental periods of 18.9 and 20.2 days when *D. tamaninii* was allowed to prey on the western flower thrips and sweet potato whitefly, respectively.

The average developmental period of *D. tamaninii* was significantly longer when fed on older individuals of *A. gossypii* at both temperatures, which can be attributed to the ease in which *D. tamaninii* can catch and feed on younger prey individuals. FAUVEL et al. (1987), studied studying the development of *Macrolophus caliginosus* WAGNER with different aphid species as prey, attributed the longer developmental period to the size, mobility and the cuticle-resistance of some aphid preys they used.

Mortality occurred mainly in the first and second nymphal instars and was generally higher when *D. tamaninii* was fed on 4-5-day-old *A. gossypii* individuals, which can be also attributed to the difficulty in catching larger prey. A total mortality of 14% was reported by ALVARADO et al. (1997) at a temperature of 25±1°C, whereby in the present study, it valued 20% and 25% at 25±1°C with 1-2- and 4-5-day-old *A. gossypii*, respectively.

The average longevity of *D. tamaninii* varied widely and was significantly longer at 18±1°C for both females and males with both prey age groups. In general, females lived longer than males when both were fed on the same prey age, while the longevity of both sexes was longer when fed on 4-5- rather than 1-2-day-old *A. gossypii*. However, FAUVEL et al. (1987) reported that the longevity of *M. caliginosus* varied widely and ranged from 39.8 to 209.7 days, which seems to have a similar trend to that of our results. No further information about the longevity was available in the literature

Temperature had an important effect on the pre-oviposition period and fecundity of *D. tamaninii*. When the predatory females were offered *A. gossypii* ad libitum as prey, they started laying eggs 4 days after adult emergence at 25±1°C, which was significantly shorter compared to that at 18±1°C, where they laid eggs after 10 days. No significant differences existed between the oviposition periods at both temperatures. Fecundity of *D. tamaninii* females was at 25±1°C with an average total progeny of 67.8 eggs significantly higher than at 18±1°C, where it was in average 11.0 egg/**&**. ALBAJES et al. (1996) reported a total number of 88 egg/**&**, when the females were fed on greenhouse whitefly on pelargonium plants. They added that fecundity might widely vary according to the plant species and prey, which may explain the difference with the fecundity found in the present work.

Although the fecundity of the predatory bug *D. tamaninii* was studied on cucumber with *A. gossypii* being offered ad libitum as prey, the effect of prey offer on the oviposition by the predatory bug is still not investigated. Therefore, the present experiments aimed to figure out the influence of fluctuating prey offer of *A. gossypii* individuals on the daily number of eggs laid by *D. tamaninii* females over their longevity. Results showed that with a fluctuating daily prey offer of *A. gossypii* individuals ranging from 7 to 70 aphid/**&**, the predatory females started laying eggs 3-7 days after their emergence and continued to lay eggs over a period from 15 to 57 days, where one *D. tamaninii* female laid in total 21-51 eggs. These values agree with those that was found in studying the fecundity of *D. tamaninii* at 25±1°C on cucumber with excess number of *A. gossypii* as prey, where a pre-oviposition and oviposition periods of to 4-6 days and 19-59 days were found, respectively (see Tab. 5). In general, increasing the number of *A. gossypii* individuals offered daily during the first 46 days of longevity had resulted in a considerable increase in the number of eggs laid daily by adult *D. tamaninii* females and vice versa, where the number of eggs laid by one D. *tamaninii* ranged from a peak of 0.7 eggs when 7 aphid/day/**&** were offered and reached up to 2.9 egg/day with a daily prey offer of 70 aphid/**&**. Starting from the 47th day, increasing the daily prey offer had not considerably enhanced the oviposition activity by the females. No previous literature was found on the effect of daily prey offer on the oviposition by *D. tamaninii*, however, in another set of experiments it was found that one *D.*

tamaninii female, when offered excess number of *A. gossypii* on cucumber at a temperature of 25±1°C, produced daily in average up to 2.9 egg/day (SALEH and SENGONCA 2001a).

The sex ratio in the progeny of *D. tamaninii* females was found to be dependent on the age of the parent females, where the percentage of females decreased as the parent females aged. Limited information were found in the literature on the progeny sex ratio of *D. tamaninii*. However, ALVARADO et al. (1997) reported a female percentage of 41.7% in 14 randomly selected *D. tamaninii* nymphs reared to adult.

Prey consumption

Successful biological control of a pest is based on the fact that the natural enemy kills a sufficient number of a prey to keep its density at a low level. As such knowledge of *D. tamaninii* is still lacking in the literature, the present laboratory experiments were conducted in order to comprehensively investigate the prey consumption by the predatory bug during its development as well as the entire longevity with two different age groups of *A. gossypii* as prey at two different temperatures.

The results of the experiments conducted on the prey consumption by *D. tamaninii* revealed that the predatory bug was able to prey successfully on both 1-2- and 4-5-day-old *A. gossypii* individuals used at higher and lower temperatures. Within the same prey age group of *A. gossypii*, higher numbers of aphids were killed daily by nymphs and adults of *D. tamaninii* at 25±1°C rather than 18±1°C. At present, there is no previous information available on the effect of temperature on the prey consumption by *D. tamaninii*. However, PERDIKIS et al. (1999) had worked on the predation rate of the similar mirid bug *Macrolophus pygmaeus* RAMBUR on *M. persicae* and reported that higher temperature had a pronounced enhancing effect on the prey consumption during development and adulthood of the predator.

Although the daily prey consumption by *D. tamaninii* at 25±1°C was higher than that at 18±1°C, the total number of *A. gossypii* from both age groups killed by *D. tamaninii* during development was much less at 25±1°C than at 18±1°C. This can be explained by that the developmental period of *D. tamaninii* with both prey age groups of *A. gossypii* at 25±1°C was only about half of that at 18±1°C (see figure 4) (SALEH and SENGONCA 2001a), so that at the latter temperature, the predatory nymphs had been consuming their prey over a much longer duration. ALVARADO et al.

(1997) reported that *D. tamaninii* during development at 25±1°C consumed a total of 127 young *A. gossypii*, which is clearly less than that found in the present study. Such a difference may be referred to that they offered daily 20 aphids for the young nymphal stages of *D. tamaninii* and 35 for the later ones, while in the present study, the daily prey offer was $\geq$30 aphids for N_1-N_3 and $\geq$70 for N_4-N_5 instars. Furthermore, they compensated for the killed aphids 4 times weekly, while in this study, new preys were added daily.

At both temperatures used, significantly higher numbers of 1-2- rather than 4-5-day-old *A. gossypii* were consumed by *D. tamaninii*. This agrees with the results obtained by SENGONCA et al. (2002), who reported that *D. tamaninii*, particularly in the early developmental stages, consumed more prey when they were offered younger *A. gossypii*. BARNADAS et al. (1998) had worked on the predatory capacity of *D. tamaninii* and *M. caliginosus* on *Bemisia tabaci* (GENNADIUS) and found that both predatory bugs, during their development and adulthood, consumed higher number of eggs rather than nymphs or adults of the whitefly.

The results of prey consumption by the adults at 25±1°C and 18±1°C showed that females had consumed significantly more *A. gossypii* from both age groups than the males. Such differentiation in the prey consumption between both sexes of *D. tamaninii* appeared with both prey ages of *A. gossypii* 3 days after adult emergence at 25°C, while after 6 and 4 days at 18°C with 1-2 and 4-5-day-old aphids, respectively. The rapid increase in prey consumption by the females at both temperatures can be explained by that they started with oviposition 4 days after their emergence at 25±1°C and 10 days at 18±1°C (SALEH and SENGONCA 2001a), which justifies a stronger need for nutrients. PERDIKIS et al. (1999) found that females of the predatory mirid bug *M. pygmaeus* consumed almost twice as much *M. persicae* as the males. LICHTENAUER and SELL (1993) had worked on the anthocorid *Orius minutus* (L.) and found that females killed 25% more *F. occidentalis* than males.

The average daily prey consumption by the successive predatory nymphal intars increased significantly with development on both age groups of *A. gossypii*. A similar trend in the prey consumption was also found with the predatory mirid bugs *Deraecoris pallens* (REUTER) (AL-HITTY et al. 1988), *Cyrtopeltis tenuis* REUTER (TORRENO and RUGUIAN 1984), *M. pygmaeus* (PERDIKIS et al. 1999) and *M. caliginosus* (FAUVEL et al. 1987) when fed on different prey species.

In a natural agro ecosystem, the number of preys available for a natural enemy will never be constant, on the contrary it will fluctuate in response to many biotic and abiotic factors. In order to be considered as a successful natural enemy, a predator is expected to be capable to adapt it self and react efficiently to such fluctuations in prey availability. The results of the experiments on the predatory efficiency of *D. tamaninii* with an alternating prey offer of *A. gossypii* showed that the daily prey consumption by the predator became higher when more preys were offered. The predatory bug consumed most *A. gossypii* individuals offered when the prey offer was 3 or 7 aphid/day, which indicates that *D. tamaninii* adults seek their prey actively and are able to adapt smoothly to a fluctuating prey availability. This confirms the conclusions made by ALVARADO et al. (1997), who reported a considerable increase in the daily prey consumption in relation to prey density.

Before considering a predator for a biological control program, it is important to investigate its affinity toward a certain developmental stage of the target pest or even the pest species to be controlled. This is true especially when we take into account that under both open field and greenhouse conditions, there are naturally several pest species, which might serve as potential prey for the predator. Therefore, more attention should be drawn to such eventual prey preference. Taking into consideration the polyphagous nature of *D. tamaninii*, it was worthy to study its prey preference and if it could survive by feeding on other alternative nutritional sources in the absence of prey.

Results of the experiments on the age preference of *A. gossypii* as prey showed that significantly higher numbers of 1-2- rather than 4-5-day-old aphids were killed by all stages of *D. tamaninii* tested. This was particularly clearer in N_3 instar, which is smaller and less vigorous than both N_5 and adult predatory individuals. FAUVEL et al. (1987) had studied the biology of the predator *M. caliginosus* with different aphid species as a prey and observed that size, mobility and cuticle-hardness of the aphid prey had an important role in the ease of predation by the polyphagous bug. This seems to apply also on the results obtained in the present experiments.

The results of the experiments on the prey species preference by *D. tamaninii* revealed that the predator was able to attack and feed on all the 5 prey species offered, i.e. *A. gossypii, M. persicae, F. occidentalis, T. vaporariorum* and *T. urticae*. All developmental stages of the predator tested, namely, N_3 and N_5 instar as well as adult female showed clear affinity toward *A.*

gossypii in regard to other prey species offered. However, considerable numbers of *T. urticae*, *F. occidentalis* and *T. vaporariorum* were also consumed. A result, which shows that despite the affinity toward the melon aphid, these 3 important pests will be also accepted by *D. tamaninii* as prey, an important fact to be considered when utilising the predator in biological control programs. Several researchers had reported that *D. tamaninii* preys on *F. occidentalis* (GABARRA et al. 1995, RIUDAVETS and CASTANE 1998), *T. vaporariorum* (GABARRA et al. 1988, CASTANE et al. 1997) and *A. gossypii* (ALVARADO et al. 1997, SALEH and SENGONCA, 2000, SENGONCA and SALEH 2002, SENGONCA et al. 2002).

No pervious information was found in the literature about the predation by *D. tamaninii* on *T. urticae*. Since the red spider mite is an important pest that occurs so frequently on cucumber and many other crops in open field and greenhouse, it is very useful to investigate if *D. tamaninii* can successfully complete its development to adult stage when exclusively offered *T. urticae* as prey. The results of this experiment showed that the spider mite was a suitable prey for *D. tamaninii* to achieve its full development in about 21 days, with a percentage mortality of 20% during development. This coincides with results obtained in studying the life table of *D. tamaninii* on young *A. gossypii* as prey, where a nymphal developmental period of about 22 days and a percentage mortality of 20% were found (SALEH and SENGONCA 2001a).

The ability of a natural enemy to feed and survive on alternative nutritional sources such as plant material may have an advantage in stabilizing its population dynamics (LALONDE et al. 1999). It was observed in the present study that the predatory bug was also able to feed on the cucumber leaf discs used in the experiments. This has sparked the interest in setting up an experiment, in order to investigate how long *D. tamaninii* would survive, when certain nutritional sources are exclusively offered to the predator to feed on. Results showed that *D. tamaninii* was able to feed and survive for considerable periods of time when fed on honey emulsion or cucumber plant. Such plant feeding habit by *D. tamaninii*, especially when prey offer was limited, had also been confirmed in other experiments conducted on the potential damage to cucumber fruits caused by *D. tamaninii* adults (see section 3.1.6). Furthermore, ALOMAR and ALBAJES (1996) reported that *D. tamaninii* was able to feed on tomato plants, particularly when the prey offer was limited.

Effect of extreme high constant and alternating temperatures

The ability of a natural enemy to adapt to different environmental conditions is an essential prerequisite for its successful utilization in a biological control program. Among other environmental conditions, temperature is considered to be a key factor affecting the biology and ecology of both harmful and beneficial insects. Taking this into consideration, the development, mortality and prey consumption by *D. tamaninii* was studied at two extreme constant temperatures of 30±1°C and 35±1°C and two alternating temperatures of 25/15±1°C and 35/22±1°C .

The results of the experiments conducted on the effect of different temperatures on the development of *D. tamaninii* showed that, the predatory bug was able to develop from egg to adult stage at the constant temperature of 30±1°C as well as both alternating temperatures of 25/15±1°C and 35/22±1°C. On the other hand, the predator was not able to achieve neither its embryonic nor nymphal development at the constant temperature of 35±1°C. In general, the average total developmental period of *D. tamaninii* became shorter with higher temperature. This agrees with the results of a previous study conducted by SALEH and SENGONCA (2001a), who reported that the predator completed its development in a significantly shorter period at a temperature of 25±1°C rather than 18±1°C.

The percentage mortality of *D. tamaninii* during its development was clearly temperature-dependent, where it was 30% at the constant temperature of 30±1°C and increased to 100% at 35±1°C. On the other hand, when the alternating temperatures of 25/15±1°C and 25/22 were used, a percentage mortality of 16.5% and 20.0% was recorded at both temperatures, respectively. This coincides with the results obtained by SALEH and SENGONCA (2001a), where a percentage mortality of 11% and 20% was recorded at temperatures of 18±1°C and 25±1°C, respectively. ALVARADO et al. (1997) reported a total percentage mortality of 14% when *D. tamaninii* was kept at a temperature of 25±1°C.

The prey consumption by *D. tamaninii* during its development and adulthood, by feeding on *A. gossypii* as prey, was considerably temperature-dependent. One *D. tamaninii* female consumed during its nymphal development at the constant temperature of 30±1°C a total of 442.2 aphids, while it consumed a total of 359.3 and 348.5 aphids at the alternating temperatures of 25/15±1°C and 35/22±1°C, respectively. On the other hand, a predatory adult female of *D. tamaninii* consumed during the first 10 days after its emergence a total of 446.4 aphid at the constant temperature of 30±1°C, while at the alternating temperatures of 25/15±1°C and 35/22±1°C it consumed a total of 267.0 and 351.5 aphids respectively. This agrees with the results obtained on the similar predatory mirid bug *M. pygmaeus* by PERDIKIS et al. (1999), who reported that the predator consumed considerably more aphids at higher temperatures. The constant temperature of 35±1°C was found to be fatal for both nymphal and adult stages of *D. tamaninii*, where the daily prey consumption started very low and decreased strongly till all predatory individuals died after a short period.

Host plant spectrum for oviposition

As the predatory bug inserts its eggs in the plant tissue, it was worthy to investigate the suitability of selected crop plant species for oviposition by *D. tamaninii*. Due to the important role a plant type plays in oviposition, these were described as host plants. In the present experiments, six different potted crop plants, i.e. tomato, tobacco, beans, sweet pepper, cucumber and eggplant were used as host plants for oviposition. Results showed that *D. tamaninii* laid eggs on all plant species tested. However, significantly higher numbers of *D. tamaninii* eggs were found on cucumber and tobacco plants, where a total of 51 and 47 egg/plant were found on both plant species, respectively. These were followed by tomato, beans, sweet pepper and eggplant, which had 44, 21, 17 and 14 egg/plant, respectively. ALOMAR et al. (1994) using a sweep net were able to sample *D. tamaninii* from 23 cultivated and non-crop plants of several families. CEGLARSKA (1999) had worked on the closely related predatory mirid bug *Dicyphus hyalinipennis* BURM. as a natural enemy in Hungarian greenhouses and reported that the highest oviposition activity of the predator was obtained on tobacco. CONSTANT et al. (1996) had used a small penetrometer to investigate the tissue hardness of plant samples in relation to oviposition by the mirid bug *M. caliginosus* and indicated that, hardness should be a choice criterion for oviposition by the predatory bug.

Interaction of *Dicyphus tamaninii* with selected beneficial arthropods

Since *D. tamaninii* is a polyphagous predator, it is possible that predation on other natural enemies might disrupt biological control and precipitate crop losses. As testing all of the potential interactions is not practical, the present study focused on three natural enemies most likely to be disrupted by a possible release of *D. tamaninii* in greenhouse. These are *A. cucumeris*, *P. persimilis* and *A. colemani* inside *A. gossypii* mummies, in the presence of unparasitized *A. gossypii* nymphs. Results showed that *D. tamaninii* mostly attacked unparasitized *A. gossypii* first. Moreover, relatively low numbers of other beneficial arthropods were attacked by *D. tamaninii*. These results indicate that this predator is unlikely to cause considerable disruption of the natural enemies tested. However, this needs to be confirmed in greenhouse. CASTANE et al. (2000) had studied the role played by *D. tamaninii* in controlling the greenhouse whitefly in tomato crops and reported that, the predatory bug consumed more greenhouse whitefly than pupae of *Encarsia Formosa* GAHAN, when both species were offered to the predator as prey in the same proportion under laboratory conditions. Moreover, GILLESPIE and MCGREGOR (2000) had studied the interaction of the closely related predatory mirid bug *Dicyphus hesperus* KNIGHT with *E. Formosa* and *P. persimilis* on tomato under greenhouse, they concluded that the effect of all natural enemies was additive and so, these natural enemies can be used in combination in framework of a biological control program.

Potential damage to cucumber fruits

Although phytophagy in some predators may have an advantage in stabilizing the predator population dynamics (LALONDE et al. 1999), some predatory mirid bugs are known to cause economic damage to crops as a result of their plant feeding habit (GABARRA et al. 1988, ALOMAR and ALBAJES 1996, COLL and RUBERSON 1998b, SAMPSON and JACOBSON 1999). Such plant feeding habit, especially when prey offer was limited, had also been observed with *D. tamaninii* during several experiments on the biology and prey consumption by the predator.

From the results of the experiments on the damage that *D. tamaninii* might possibly cause to cucumber fruits, it is clear that the number of feeding punctures on cucumber fruits was significantly reduced by the presence of the leaf and prey. This agrees with the results obtained by ALOMAR and ALBAJES (1996), who reported that the damage to tomato fruits caused by *D. tamaninii* occurs only when the populations of prey species decline. On the other hand, MCGREGOR et al. (2000) had worked on the potential damage caused by the omnivorous mirid bug *D. hesperus* to tomato fruits in the presence and absence of leaves and prey, they found that substantial feeding on fruits took place only when leaves were not provided. They added that the absence of prey (*E. kuehniella* eggs) did not have significant influence on the fruit feeding by the predator.

In order to investigate if leaf or fruit o f cucumber would be preferred by *D. tamaninii*, when both were simultaneously offered, experiments were set up, in which the probability that the predatory females would be encountered on leaf, fruit or elsewhere in the experimental cage was quantified. Twenty-four hours prior to the experiments, *D. tamaninii* females had access either to excess *A. gossypii*, cucumber leaves or were left starved. Regardless the food treatment prior to the experiments, all *D. tamaninii* females were generally observed more often on cucumber leaves than on fruits. This might be explained by that the leaves, being possibly a better habitat for oviposition, were more preferred by the females to rest on. These results are consistent with the results obtained by MCGREGOR et al. (2000), who conducted similar research on tomato leaf-fruit preference of *D. hesperus* and reported that, the probability that the insects would be found on leaves or fruit increased with time. Moreover, the starved *D. hesperus* individuals were more likely to be encountered on leaves than other individuals.

As the results of the present study showed that *D. tamaninii* prefers leaves to cucumber fruits, the potential damage to cucumber fruits by the phytophagous predator remains low. This confirms the observations made by GABARRA et al. (1995), who studied *D. tamaninii* as a predator of greenhouse whitefly and western flower thrips on cucumber and reported that under greenhouse conditions, the predator caused no damages to cucumber fruits.

Greenhouse experiments

Trials were performed under greenhouse conditions in order to study the efficiency of *D. tamaninii* in reducing *A. gossypii* populations on cucumber under more natural conditions. Two release timings of *D. tamaninii* were tested, where 42 adult predators, were released in the cabins, one week (cabin I) and two weeks (cabin II) after infesting the cucumber plants with *A. gossypii*.

Results showed that *D. tamaninii* was able to successfully feed, reproduce and establish its population on cucumber under greenhouse conditions with *A. gossypii* as prey. The ability of *D. tamaninii* to successfully feed and achieve its full development on *A. gossypii* as prey was documented under laboratory conditions in several previous studies (SALEH and SENGONCA

2000, 2001a, b, SENGONCA and SALEH 2002), where one adult *D. tamaninii* female consumed up to 60 *A. gossypii* individuals (SENGONCA et al. 2002). The results of the present study showed that up to 90% reduction in the population of *A. gossypii* was achieved when 2 adult *D. tamaninii* individuals were released per cucumber plant. CASTANE et al. (1996) had tested the efficiency of *D. tamaninii* in reducing the population of the western flower thrips *F. occidentalis* on cucumber under greenhouse conditions. They reported that a release r atio of 1:10 predator:prey was enough to achieve an acceptable control level when the initial thrips population was low at the beginning of the season (1 adult/leaf). On the other hand, a release rate of 3:10 predator:prey was necessary when the initial thrips population was 5 adult/leaf. *D. tamaninii* was also successful in reducing *T. vaporariorum* populations on tomato, where more than 60% reduction in the nymphal and adult stages of the whitefly was achieved, when the predator was released in a ratio 3:10 predator:prey (CASTANE et al. 1996).

Although the numbers of *A. gossypii* in all experimental cabins began to decline starting from the 4^{th} week after infestation with the aphid, *D. tamaninii* numbers in cabin I and II maintained their increasing tendency till the end of the experiments. Similar trend in the number of *D. tamaninii* was also found by GABARRA et al. (1995), who studied the population development of the predator and reported that no significant differences were found in the numbers of *D. tamaninii* at the medium or high availability of the prey. On the other hand, they added that *D. tamaninii* was barely able to reproduce on cucumber in the absence of prey.

The efficiency of *D. tamaninii* in reducing the population of *A. gossypii* was generally higher when the predator was released 1 week rather than 2 weeks after infestation with *A. gossypii*, where a reduction percentage of 78% and 65% were achieved in the 3^{rd} week at both release timings, respectively. This indicates that an early release of the *D. tamaninii*, while the aphid pest pressure is still low, would be more effective in its control. SAMPSON and KING (1996) had introduced the similar predatory mirid bug *M. caliginosus* against the whitefly on tomato in 24 Dutch greenhouses at different release timings and rates, they reported that such introductions were most effective when made early in the season with a minimum release rate of 1 predator/m^2.

The behavioural observations on the movement and location of *D. tamaninii* adults among the cucumber plants within the glass cabins, revealed that the predator searches its prey actively where higher numbers of the predator were usually encountered on the plants or the plant parts with higher prey density. This is consistent with the results obtained by MONTSERRAT et al. (2000), who reported that on the contrary of *M. caliginosus*, *D. tamaninii* left the plant when the prey density became low.

Conclusion

D. tamaninii was able to successfully feed, develop and reproduce when exclusively fed on *A. gossypii* as prey at a wide range of low and high temperatures, however a constant temperature of 35±1°C or higher will be fatal to the predator. The predator can smoothly adapt to fluctuating prey offer and will maintain its capability of oviposition even when the prey is relatively scarce. Moreover, the predatory bug seems to occupy a wide plant host range for oviposition. The prey preference experiments indicated that, although *D. tamaninii* showed a clear affinity toward *A. gossypii*, it accepted also other important pest species offered as prey. Moreover, the predatory bug, if being released with certain natural enemies, is not likely going to disrupt their affectivity in a biological control program. Despite the plant feeding habit of *D. tamaninii*, the potential damage to cucumber fruits caused by the bug remains low. The release of 2 *D. tamaninii* adults per cucumber plant in greenhouse was sufficient to achieve up to 90% reduction in *A. gossypii* population. Nevertheless, it is speculated that an earlier release of *D. tamaninii* would be more effective in the biological control of *A. gossypii*.

The present study has revealed that *D. tamaninii* seems to be a very promising natural enemy for the biological control of the melon aphid on cucumber. However, there are still some points to be further investigated, for example, the suitability of the predator for the biological control of white fly, thrips and spider mite on different crops, the interaction between *D. tamaninii* and other natural enemies under green house conditions and establishing a feasible method for the mass rearing of the predator.

SUMMARY

The present research aimed to study the biology and prey consumption by the polyphagous predatory bug *Dicyphus tamaninii* WAGNER (Heteroptera: Miridae) during its development and longevity by feeding on different age groups of the melon aphid *Aphis gossypii* GLOVER (Homoptera: Aphididae) as prey at two constant temperatures of 25±1°C and 18±1°C as well as different extreme high constant and alternating temperatures. The prey preference and alternative nutritional sources for the predator as well as the host plant spectrum for oviposition were also investigated. Moreover, experiments were conducted to investigate the interaction of *D. tamaninii* with other natural enemies and the potential damage it might cause to cucumber fruits under different nutritional conditions. Finally greenhouse trials were carried out in order to confirm the efficiency of *D. tamaninii* for the biological control of *A. gossypii* on cucumber under greenhouse conditions.

The results of the laboratory experiments on the biology of *D. tamaninii* showed that the predatory bug was able to successfully feed, develop and reproduce when exclusively fed on either 1-2- or 4-5-day old *A. gossypii* as prey at a wide range of low and high temperatures. However, a constant temperature of 35±1°C was fatal to the predator. The total developmental period from egg to adult stage with both prey age groups of *A. gossypii* varied widely according to temperature, where it was at 25±1°C in average 33.6 (**&&**), 34.6 (**%%**) days, when the predator was fed on 1-2-day-old *A. gossypii* individuals as prey. The total mortality during development from N_1 instar to adult valued at 25±1°C a percentage of 20%. Longevity of *D. tamaninii* varied widely and was at 25±1°C for females in average 29.0 days, while for males 26.3 days. Adult females began oviposition 4 days after emergence at a temperature of 25±1°C with an average total progeny of 67.8 egg/**&**. Results showed that with a fluctuating prey offer between 7 and 70 *A. gossypii* /day/**&**, adult *D. tamaninii* females started laying eggs 3-7 days after their emergence. One *D. tamaninii* female laid in total 21-51 eggs over an oviposition period ranging from 15-57 days. The percentage of females in the total progeny of *D. tamaninii* was 56% in the 1^{st} week and decreased gradually as the parent females aged, where it approached nil in the 9^{th} week.

D. tamaninii consumed during its development from N_1 to the adult stage significantly more 1-2- rather than 4-5-day-old *A. gossypii* individuals, where it consumed at 25±1°C in average 313 (**&&**), 301 (**%%**) aphids and 166 (**&&**), 143 (**%%**) aphids of both age groups, respectively. The prey consumption by *D. tamaninii* adults over their entire longevity showed according to temperature, prey age group and sex of the predator significant variations. For example when 1-2-day-old *A. gossypii* individuals were offered, the total prey consumption was at 25±1°C in average 844 (**&&**), 494 (**%%**) aphids. In multi-choice trials, the predator showed clear affinity

toward *A. gossypii* in regard to other prey species offered as prey. However, considerable numbers of *Tetranychus urticae*, *Frankliniella occidentalis* and *Trialeurodes vaporariorum* were also consumed. *Myzus persicae* was the least preferred by all predatory stages tested. In a separate experiment, *D. tamaninii* was able to complete its development from N_1 to adult stage in an average of 21 days, with 20% mortality, when fed exclusively on *T. urticae* as prey. Furthermore, results showed that *D. tamaninii* females were able to survive for an average of 41.2, 14.5, 9.8 and 3.8 days when exclusively offered *A. gossypii*, honey emulsion, a cucumber plant or left starved, respectively.

D. tamaninii was able to lay eggs on a wide range of plant species. However, it was clear that among the six plant species used, cucumber, tobacco and tomato, had significantly the highest counts of *D. tamaninii* eggs.

The interaction of *D. tamaninii* in with *Amblyseius cucumeris*, *Phytoseiulus persimilis* and *Aphidius colemani* (*A. gossypii* mummies) in the presence of unparasitized *A. gossypii* nymphs. Was studied in the laboratory. Results showed that *D. tamaninii* mostly attacked unparasitized *A. gossypii* first. Moreover, only an average of 3.8, 2.6 and 0.2 individual/day/**&** were attacked from *A. cucumeris*, *P. persimilis* and *A. colemani*, respectively.

The results of the experiments on the potential damage to cucumber showed that the number of feeding punctures on the fruits was significantly reduced by the presence of the leaf and prey. Moreover, *D. tamaninii* preferred leaves to cucumber fruits, a fact that leads to the conclusion that the potential damage to cucumber fruits by the phytophagous predator remains low.

The results of the greenhouse experiments showed that the efficiency of *D. tamaninii* in reducing the population of *A. gossypii* on cucumber was generally higher when the predator was released 1 week rather than 2 weeks after infestation with *A. gossypii*, where a percentage reduction of 78% and 65% were achieved in the 3rd week and reached to 87% and 84% in the 5th week at both release timings, respectively.

In conclusion, *D. tamaninii* exhibited under laboratory as well as greenhouse conditions many features, which make it a very promising natural enemy to be used in framework of a biological control program of the melon aphid on cucumber. However, there are still some points to be further investigated, for example, the suitability of the predator for the biological control of white fly, thrips and spider mite on different crops, the interaction between *D. tamaninii* and other natural enemies under green house conditions and establishing a feasible method for the mass rearing of the predator.

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Acknowledgments

My profound thanks and indebtedness are due to Prof. Dr. Cetin Sengonca, my “Doktorvater”, who has given this word its real meaning, through his guidance, encouragement, inspiration and positive criticism over the entire work.

Without any reservation, I would like to thank Prof. Dr. G. Noga for kindly using his valuable time as a co-referent for the dissertation.

My thanks are extended to Prof. Dr. O. Alomar, IRTA, Centre de Cabrils, Barcelona, Spain, for providing the individuals of *D. tamaninii* used to initiate the stock culture for the present research.

Sincere acknowledgments are due to Dr. rer. nat. Richard Haverkamp for his friendly help in the statistical analysis of the results.

I wish also to accord a special debt of gratitude to all my colleagues in the department of entomology and plant protection as well as in the other departments of the institute for phytopathology, for the unforgettable friendly research environment they have created, and the cooperative spirits they have shown at each single step of the research and writing of thesis.

This research could have never been done without the generous financial support from the German Academic Exchange Service “DAAD”.

Finally, special thanks and gratitude are due to Rajaa, my wife, who, in addition to her endless support and encouragement, has been always solving the computer-related problems I faced during the study.