Daily Consumption and Predation Rate of Different *Stethorus punctillum* Instars Feeding on *Tetranychus urticae*

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Laboratory experiments were conducted to evaluate the prey stage preference and the daily consumption of each stage of the coccinellid predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) feeding on the two-spotted spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae). Groups of different life stages of the prey were offered (eggs, larvae, nymphs and adults). The prey preference varied with the stage of *S. punctillum*. First larval instars had no significant preference among the *T. urticae* stages offered. Second larval instars consumed significantly more spider mite larvae in comparison with nymphs. In contrast, third larval instars indicated a strong preference for mite eggs. Significantly fewer *T. urticae* larvae were consumed by the fourth larval instars of *S. punctillum*, in comparison with the three other mite stages. Finally, adult predators consumed significantly more mite eggs than the other stages offered. This preferential trend was similar for all adults tested, whether during the pre-oviposition or the oviposition period.
KEY WORDS: *Stethorus punctillum; Tetranychus urticae;* daily consumption; prey stage preference.

INTRODUCTION

Tetranychus urticae (Koch) (Acari: Tetranychidae) is considered to be the most polyphagous species of the Tetranychidae. It is a major pest of vegetables and ornamentals in greenhouses and of several other agricultural outdoor crops (9,24,30). This species is adapted to various environmental conditions and is distributed worldwide, causing loss of quality and yield or the death of the plants by sucking out the contents from the leaf cells (8,9,24). However, there are several important predators of *T. urticae* (3,9). All known species of the genus *Stethorus* Weise (Coleoptera: Coccinellidae) are predators of spider mites (1-3,7,11,15) and several of them have been suggested to have potential as biological control agents of spider mites in agricultural crops (12,13,22,25). *S. punctillum* is a Palaearctic species first found in North America in the 1940s and was identified as one of the most important predators of tetranychid mites in peach orchards (20). This species is now released in North America to control *T. urticae* (21). Most

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studies concentrate on the efficacy of *Stethorus* spp. in orchards (6,7,13,20,25), whereas little research has been done on biocontrol in greenhouses. Rott and Ponsonby (23) examined the predatory behavior and activity of *S. punctillum* larvae on *T. urticae* and its response to environmental factors and host plant species under glasshouse conditions. Furthermore, Rott and Ponsonby (24) examined the predatory behavior of *S. punctillum* and *Amblyseius californicus* McGregor (Acari: Tetranychidae) on *T. urticae* and assessed the way each species responded to environmental factors and host plant species under greenhouse conditions. Raworth (21) studied larval voracity, the lower developmental temperature threshold and the developmental time of *S. punctillum.* Roy *et al.* (26) investigated the thermal characteristics of *S. punctillum* and its prey *Tetranychus mcdanieli* McGregor (Acari: Tetranychidae). However, given that this species may have certain advantages as a potential biocontrol agent against spider mites (24), further experimental work is essential, in order to assess the feasibility of its use for mite control. In our study, we examined the daily consumption and prey preference of larval and adult *S. punctillum* on *T. urticae.*

MATERIALS AND METHODS

Plant material Twenty-four 10-cm pots were sown each with six seeds of French bean (*Phaseolus vulgaris* L.) at a depth of 1 cm in peat-based pottery compost. After the plants had reached a height of 30 cm they were transferred to Plexiglas cages with a metal tray as a base and a nylon net in the front. Broad bean plants (*Vicia faba* L.) were sown in the greenhouse for use as plant material for *T. urticae* and *S. punctillum.*

***T. urticae* and *S. punctillum* culture** The French bean plants established in the Plexiglas culture cages were artificially infested with *T. urticae* individuals. After 3–8 days *T. urticae* was established in the cages and it was kept until the end of the experiment. *S. punctillum* adults were individually placed in 275×150×100 mm Plexiglas boxes with two holes covered with a fine net for sufficient ventilation. The boxes were lined with well-wetted paper and on top of that broad bean leaves were placed with the upper side facing down. French bean leaves infested with *T. urticae* were carefully brushed in the box to provide food to *S. punctillum* every second day and the wetted paper was changed weekly.

Experimental cages Plexiglas cages were used for the observation of daily consumption by *S. punctillum.* Each cage was made from a Plexiglas sheet (75×38×8 mm) with a central hole 24 mm in diameter. This was placed between a 75×38×3 mm Plexiglas sheet with a hole 10 mm in diameter covered with a fine net cloth to provide sufficient ventilation and a glass sheet with the same dimensions. A piece of moist filter paper was placed between the glass slide and the Plexiglas sheet and beneath a broad bean leaf (27). The leaf was changed every second day. French bean leaves infested with *T. urticae* were brushed first on a petri dish lined with a filter paper and then transferred to the Plexiglas cage. Finally, one *S. punctillum* individual was released every time in each cage. A 50-mm-diam petri dish was used for observing mating of *S. punctillum* and studying consumption during oviposition. A hole (28 mm in diameter) was made in the middle of the lid and was covered with a fine mesh cloth. A well-wetted cotton ball was placed inside the petri dish and a broad bean leaf with the upper side facing down was placed on the cotton. *T. urticae* individuals were placed on the broad bean leaf by brushing them from heavily infested French bean leaves.

Daily consumption counts Daily consumption by *S. punctillum* was studied by putting a constant number of *T. urticae* individuals in each cage. For this purpose, 50 eggs, 30 larvae, 30 nymphs and 30 adults were offered to each *Stethorus*. Daily consumption was calculated by subtracting the number of *T. urticae* individuals left from the number of individuals brushed in the cage. Consumption of *T. urticae* by *S. punctillum* was studied throughout the whole life cycle comprising the four larval stages, and the adult females before and during oviposition. *T. urticae* were placed first in the cage, and then a single *S. punctillum* larva was released and the cage was closed. The sandwich boxes were placed in incubators set at 24±2°C, 18L:6D and 75% r.h. (obtained by using saturated sodium chloride). The number of *T. urticae* consumed by *S. punctillum* was examined under a microscope after 24 h. *S. punctillum* was transferred to new cages the following day and this procedure was repeated until its pupation period. Consumption by the adult stage in the pre-oviposition period was studied in the same way as with larvae. After that the adults were placed in petri dishes to enable them to mate. Each experiment was replicated nine times. For statistical analysis – since Levene tests were >0.05 (in all cases d.f.=3, 32) and suitable transformations were not available – the data were submitted to a Kruskal-Wallis analysis of variance (in all cases d.f.=3), using the statistical package JMP (28). Means were separated using the Tukey's Multiple Comparison Difference at P=0.05 (29). In the other cases, where Levene tests were <0.05 or suitable transformations were possible, the data were submitted to one-way analysis of variance, and means were separated by the Tukey-Kramer HSD test, at P=0.05 (28).

TABLE 1. Daily consumption (mean ± S.E.) in actual numbers of *Tetranychus urticae* eggs, larvae, nymphs and adults by each stage of *Stethorus punctillum*

S. punctillum stage	Daily consumption of *T. urticae*			
	Egg	Larva	Nymph	Adult
First instar larvae	16.67±3.20a	18.56±1.94a	19.56±1.76a	14.33±2.25a
Second instar larvae	16.86±4.44ab	20.86±2.08a	14.43±2.72b	16.86±1.08ab
Third instar larvae	34.43±2.78a	23.00±0.90b	21.93±1.10b	20.79±1.45b
Fourth instar larvae	15.20±3.53a	9.60±2.29b	18.40±2.29a	19.00±2.95a
Adults (pre-oviposition period)	38.30±1.75a	24.67±0.91b	22.67±1.06b	22.18±0.94b
Adults (oviposition period)	46.17±1.42a	28.33±0.95b	21.17±4.04b	20.33±4.17b

Within rows, means followed by a common letter do not differ significantly; Tukey's multiple comparison difference, or Tukey-Kramer HSD test at P=0.05.

RESULTS

Prey preference differed significantly among developmental stages of *S. punctillum* (Table 1). No significant differences were noted between different *T. urticae* stages consumed by first larval instars of *S. punctillum* (Levene's test F=1.85, P=0.1570; K-W ANOVA χ^2=6.21, P=0.1014). In contrast, significant differences were noted in the case of the second larval instars (Levene's test F=1.18, P=0.3317; K-W ANOVA χ^2=12.19, P=0.0067). Significantly more *T. urticae* larvae than nymphs were consumed by the second instar larvae of *S. punctillum*. For the third larval instar significant differences were also found (Levene's test F=1.82, P=0.1618; K-W ANOVA χ^2=19.48, P=0.0002). At that stage, significantly more spider mite eggs were consumed as compared with the three other stages offered. Finally, for the last (fourth) larval instar of *S. punctillum*, significant differences were noted among the different mite stages consumed (Levene's test F=6.06, P=0.0022; one-way ANOVA d.f.=3.32, F=12.92, P<0.0001). In that case, mite larvae were found to be the least preferable stage.

Stethorus punctillum adults consumed 75% or more of the prey offered in the pre-oviposition period (Table 1). Also, significant differences were recorded among the *T. urticae* stages consumed (Levene's test F=2.15, P=0.1122; K-W ANOVA χ^2=18.31, P=0.0004). Beetle adults at that stage indicated a significant preference for spider mite eggs. In addition, significant differences were also noted for prey-stage preference for coccinellid adults during the oviposition period (Levene's test F=0.67, P=0.5748; K-W ANOVA χ^2=29.91, P<0.0001). As above, significantly more mite eggs were consumed than other mite stages.

DISCUSSION

Daily consumption, as a fraction of *T. urticae* prey offered, tends to be higher in the adult stage than in all larval stages of *S. punctillum*. Hence, for all prey stages combined, adults consumed more than three-quarters of the *T. urticae* items offered, while the respective figure for larval stages of *S. punctillum* was only 55%. In general, it is well established that most *Stethorus* species are considered to be 'high density predators', since they require abundant prey (15,16,20). The high rate of *T. urticae* consumption by *S. punctillum* adults suggests that this species has certain advantages as a potential biocontrol agent, due to their dispersal characteristics during augmentative releases of this species. In addition, the adults of *Stethorus* spp. are long-lived (20), which conveys an additional advantage. According to our results, there is an identical prey-stage preference of adults in the pre-oviposition and oviposition periods. Thus, we can conclude that *S. punctillum* adults consume more mite eggs than other mite stages, and this trend is similar for sexed or unsexed adults. This strong preference may be attributed to nutritional characteristics related to the composition of *T. urticae* eggs, or is part of the prey-seeking behavior of *S. punctillum* adults. Further experimental work is needed to examine the basis of these hypotheses.

The findings of the present study show that prey preference varies remarkably among the larval stages of *S. punctillum*. For the first larval instar of the predator, no specific preference was recorded among the four *T. urticae* stages offered. Putman (20) stated that first larval instars of *Stethorus punctum* (LeConte) (Coleoptera: Coccinellidae) fed largely on eggs and smaller nymphs of *Tetranychus bimaculatus* Harvey (Acari: Tetranychidae), *Paratetranychus ununguis* (Jacobi) (Acari: Tetranychidae) and *Paratetranychus* sp. The second instar larvae of *S. punctillum* showed an equal preference for mite larvae, eggs and adults, all of which were preferred to nymphs. As in the case of adults, third larval instars of *S. punctillum* showed a significant preference for *T. urticae* eggs. Finally, the fourth instar of *S. punctillum* showed an equal preference for mite eggs, nymphs and adults. Collyer (5) reported that all *S. punctillum* instars showed a definite preference for larger *Metatetranychus ulmi* (Koch) (Acari: Tetranychidae) stages infesting fruit orchards in southeastern England. We can assume that, as *S. punctillum* develops from the third larval instar to the adult stage, an 'egg preference' is gradually established, despite the fact that there is an equal preference for eggs and other mite instars for old (fourth instar) larvae. Moreover, the number of spider mites consumed daily by *S. punctillum* larvae increased with the instar (with the exception of nymphs) until the fourth instar, where this rate was reduced. This could be attributed to the fact that the fourth larval period includes a pre-pupal period of about one day in which *S. punctillum* does not eat at all (4,10,20). This 'low' consumption rate may be the main reason for the non-existence of the aforementioned 'egg preference' at that stage. Nevertheless, according to Raworth (21), the number of *T. urticae* eggs consumed by *S. punctillum* larvae increased with the instar. Generally, any knowledge about the prey-stage preference of this species can be utilized in practice in augmentative releases for spider mite control, since it denotes that a specific developmental stage of the predator – when the majority of the prey items are in a more preferable stage for predation – should be released.

Biological control of *T. urticae* in glasshouses is based currently on regular curative releases of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (23, 24). However, the response of this species can be negatively affected by several factors (17-19). *S. punctillum* is considered a promising biological control agent against *T. urticae* in glasshouses (24). Apart from glasshouses, *S. punctillum* has effectively controlled spider mite populations on cotton and vineyards in Europe (14) and on citrus in Japan (31). This species is a voracious predator and is able to seek locally isolated outbreaks of mites (24) which may not be controlled by regular releases of predatory mites. The adult voracity suggests that the addition of a winged, long-lived predator that is able to locate 'hot spots' of spider mites rapidly would be useful in developing a biocontrol-based pest management program.

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