Feeding Potential of *Nesidiocoris tenuis* (Reuter) on the Twospotted Spider Mite, *Tetranychus urticae* Koch, under Laboratory Conditions[1]

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ABSTRACT *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important insect pests of tomatoes in greenhouses as well as open cultivation throughout the world. *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is a polyphagous predatory bug, which is commonly found in tomato ecosystems. The present study was aimed at studying the predatory potential of *N. tenuis* against *T. urticae.* Results of this study indicate that all stages of the bug are highly predacious against *T. urticae.* Male nymphs consumed 11.8, 55.5, 66.3, 75.3, and 78.3 mites at the rate of 5.9, 18.5, 33.2, 37.6, and 39.1 mites per day for instars 1–5, respectively. The rate of consumption by female nymphs was 15.9, 61.8, 81.1, 88.8, and 90.1 mites at the rate of 7.9, 20.6, 40.6, 44.4, and 43.0 mites per day for instars 1–5, respectively. The overall consumption by female bugs (1137–1430 mites) was also higher than that of males (858–1092 mites). Augmentative releases of later instar nymphs of *N. tenuis* against *T. urticae* might therefore assure better mite control.

The twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), with its high development rate and fecundity, is one of the most destructive pests of many crops worldwide (Helle & Sabelis 1985, Danks 2006, Yang 2013). This species feeds on host leaf tissue and removes cell contents (Tomczyk & Kropczyńska 1985), resulting in the destruction of chloroplasts, loss of leaf chlorophyll, and reduction in the net photosynthetic rate (Sances et al. 1981, De Angelis et al. 1982, Park & Lee 2002). Mite injury results in yellowing, discoloration, and ultimately bronzing of leaves that can severely affect plant physiological processes, leading to reduction in growth, flowering, and yield (Tomczyk & Kropczyńska 1985). Farmers mainly rely on chemical pesticides to control this pest; however, long-term and over-dosage applications of chemical pesticides usually result in food safety concerns, resistance to pesticides, and adverse effects on non-target organisms (Liburd et al. 2007, Van Leeuwen et al. 2010, Attia et al. 2013). Ecologically-based methods to reduce mite injury in agricultural practices are essential for integrated pest management (Wang et al. 2003, Alzoubi & Çobanoğlu 2010). Many predacious natural enemies, such as predatory mites, acarophagous thrips, ladybird beetles, and predacious anthocorids (Hoy 2011), are known to be highly effective against herbivorous spider mites.

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Specialist predators have a high degree of prey specificity and are considered appropriate to reduce a specific pest population below economic injury level (Coppel & Mertins 1977). Historically, the view among biological control practitioners was that generalist predators are less important than specialist predators, but there are reports (Edwards et al. 1979, Chiverton 1986, Symondson et al. 2002), which show that generalist predators in certain manipulated systems could be as important as specialist predators in other systems. These generalist predators include carabids (Lövei & Sunderland 1996), coccinellids (Cottrell & Yeargan 1998), neuropterans (Jacometti et al. 2010), predatory hemipterans (Stoner et al. 1975), and predatory mites (Rhodes et al. 2006). A major advantage of using generalist predators is their ability to control multiple insect pests and survive on plant material or alternative prey even when their major prey is scarce or absent (Wyss 1995, Jacometti et al. 2010). Although generalist predators have this advantage, they generally have different degrees of acceptance for various types of prey (Richards 1982). Prey characteristics, such as host breadth, prey chemistry, and prey behavior and morphology, can play important roles in the acceptance of prey (Dyer & Floyd 1993). For example, predatory hemipterans are known to exhibit prey preferences based on differences in prey mobility (Eubanks & Denno 2000), size (Cogni 2002), and prey species (Foglar et al. 1990). In addition to prey acceptance, understanding feeding capacity of different stages of generalist predators is crucial to aid mass rearing and release in biological control programs. Because younger predators tend to feed less than mature predators (Prieto et al. 2016), quantification of feeding potential of younger stages of a predator relative to older ones will help determine the number of predators necessary to release to control pests in the field and help researchers decide the optimal amount of prey needed for mass rearing of the predators.

The tomato mirid bug, *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), is a generalist predator that feeds on various pests, including whiteflies and leafminers (Urbaneja et al. 2005, Sánchez & Lacasa 2008, Perdikis et al. 2009, Hughes et al. 2009). This zoophytophagus bug, commonly found on tomatoes, is able to complete development on *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and *T. urticae* (Urbaneja et al. 2003). Feeding potential describes how much food is required for each stage of the predator, and is an important measure to determine the effectiveness of the predator against a given prey.

This study explores in greater detail how feeding changes occurred for each stage when the predators were fed a single prey species. No study has been conducted previously on feeding potential of *N. tenuis* on *T. urticae*. The aim of this study was to compare male and female *N. tenuis* as biocontrol agents of *T. urticae* by evaluating their feeding potential using per capita consumption of prey. We therefore tested how many prey were required to complete each stage. This study is therefore conducted to investigate the feeding potential of different stages of *N. tenuis* against *T. urticae* for use as an augmentative biological control agent.

Material and Methods

This study was conducted in the biocontrol research laboratory of the Department of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India (30.01 N. latitude, 77.00

E. longitude). An experiment was conducted to quantify daily feeding amount by different stages of *N. tenuis* against *T. urticae*.

Rearing of *T. urticae* and *N. tenuis*. *T. urticae* were obtained from a stock culture from tomato plants that has been maintained in the laboratory since 2005. This colony was reared and maintained on tomato plants raised in pots in a climate chamber at 25±1°C and a 12:12 (L:D) h photoperiod. Mites were reared on tomato plants for two generations before being used for experiments.

Similarly, adults of *N. tenuis* were obtained from a stock culture which was maintained on tomato plants by providing *T. urticae* as host. These adults were released on the tomato plants infested with *T. urticae* and reared for one generation at 25±0.5°C and 70±5% R.H. in the bio-control laboratory before being used for experiments.

Bioassasys. To study the predatory potential of *N. tenuis*, 40 newly emerged individuals of the same stage were moved individually to a single tomato leaf in a glass test tube (16×150 mm) having a predetermined number (30 to 80) of adult mites. In order to select adult mites, tomato leaves were carefully examined under the stereo zoom binocular microscope (SZ2, Olympus, Tokyo, Japan) and the unwanted stages of the prey were removed carefully with the help of a fine entomological needle. The petiole of the leaf was wrapped with moist cotton to maintain the turgidity of the leaf. A single predator was released in each test tube containing an excised leaf with prey. Tubes were incubated at 25±0.5°C, 70±5 % R.H, and 12:12 (L:D) h photoperiod. The number of mites consumed by each individual predator was counted and fresh prey were given every 24 h until they entered the next stage. After emergence of the adults, they were anaesthetized and sexed under a dissecting microscope. Because individuals were reared in separate test tubes, the prey consumed by different stages of male and female individuals were recorded separately. Each treatment was replicated 19 times for females and 21 times for males.

Data analysis. The data obtained were analyzed using one-way analysis of variance (ANOVA) using the SPSS v16.0 (SPSS Inc., Chicago, IL) statistical program (SPSS 2015). Significant differences were separated using multiple mean comparisons by Duncan's Multiple Range Test (DMRT, $P < 0.05$) (Duncan 1955). T-tests were used to compare differences between male and female feeding potential.

Results

DMRT analysis showed that the number of mites consumed significantly increased at each successive predatory instar ($F = 918.6$; df = 4, 100, $P < 0.001$). Significant differences in consumption rates of mites were observed among all instars of *N. tenuis* males (Table 1). Male nymphs consumed a mean of 11.8, 55.5, 66.3, 75.3, and 78.3 mites for instars 1–5, respectively, and these values were statistically significantly different. Male nymphs consumed a total of 287.2 mites during their entire nymphal period, whereas, adults consumed a total of 779.1 mites. Each instar consumed 5.9, 18.5, 33.2, 37.6, and 39.1 mites per day, respectively, which differed significantly (Table 1). During the adult stage, *N. tenuis* males consumed 33.8 mites per day.

The rate of consumption by female *N. tenuis* nymphs was 15.9, 61.8, 81.1, 88.8, and 90.1 mites for instars 1–5, respectively. The feeding potentials of female

Table 1. Feeding potential of male *Nesidiocoris tenuis* on *Tetranychus urticae.*

	Number of mites consumed/instar		Number of mites consumed/day	
Stage (Instar)	Mean ± SE	Range	Mean ± SE	Range
1st instar	11.8 ± 1.1 e[a]	10–15	5.9 ± 0.8 e[a]	5.0–7.5
2nd instar	55.5 ± 2.0 d	50–64	18.5 ± 1.2 d	16.7–21.3
3rd instar	66.3 ± 2.3 c	61–83	33.2 ± 1.6 c	30.5–41.5
4th instar	75.3 ± 2.2 b	70–89	37.6 ± 1.6 b	35.0–44.5
5th instar	78.3 ± 2.0 a	72–88	39.1 ± 1.4 a	36.0–44.0
Consumption during nymphal stage	287.2 ± 3.8	270–334	26.1 ± 1.2	24.6–30.4
Consumption by adults	779.2 ± 4.0	740–800	33.9 ± 0.8	32.2–34.8
Total consumption	1056.5 ± 6.9	858–1092	31.1 ± 1.2	25.2–32.1

[a]Means in the same column followed by a common letter are not significantly different by DMRT ($P = 0.05$).

fourth and fifth instars on mites were statistically similar to each other (Table 2). Over their lifetime, female nymphs and adults of *N. tenuis* consumed 337.7 and 989.5 mites, respectively. Female nymphs consumed 7.9, 20.6, 40.6, 44.4, and 43.0 nymphs per day for instars 1–5, respectively (Table 2). Adult females consumed 33.8 *T. urticae* mites/day, whereas, over the total life span, *N. tenuis* fed on 31.0 mites/day. Consumption rate by the first three female instar *N. tenuis* were significantly different ($P < 0.01$), whereas, per day consumptions of *T. urticae* by fourth and fifth instars of *N. tenuis* were statistically similar ($P = 0.05$) (Table 2).

Table 2. Feeding potential of female *Nesidiocoris tenuis* on *Tetranychus urticae.*

	Number of mites consumed/instar		Number of mites consumed/day	
Stage (Instar)	Mean ± SE	Range	Mean ± SE	Range
1st instar	15.9 ± 1.3 d[a]	13–19	7.9 ± 0.9 d[a]	6.5–9.5
2nd instar	61.8 ± 2.1 c	54–67	20.6 ± 1.2 c	18.0–22.3
3rd instar	81.1 ± 1.9 b	74–86	40.6 ± 1.3 b	37.0–43.0
4th instar	88.8 ± 1.7 a	83–93	44.4 ± 1.2 a	41.5–46.5
5th instar	90.1 ± 1.5 a	86–95	43.0 ± 1.0 a	42.0–47.0
Consumption during nymphal stage	337.7 ± 2.6	322–347	29.2 ± 0.8	30.7–31.6
Consumption by adults	989.5 ± 7.6	807–1087	38.4 ± 1.5	31.0–41.8
Total consumption	1132.0 ± 7.8	1137–1430	37.0 ± 1.3	31.6–39.7

[a]Means in the same column followed by a common letter are not significantly different DMRT ($P = 0.05$).

Fisher's t-test analysis demonstrated differences in the male and female feeding potential. The feeding potential of first, third, and fourth instars of male and female differed significantly as the Fisher's t values were 3.05, 2.36, 2.03, respectively, which were higher than the tabulated values (2.02) at $P=0.05$. Similarly, the feeding potentials of adult males and females of *N. tenuis* against *T. urticae* also varied significantly (t= 2.90; higher than tabulated value t= 2.02 at $P=0.05$), however, no statistical differences were observed in second (t=1.18) and fifth instars (t=1.68) of male and female nymphs, as well as for total nymphal instars (t=1.94).

Discussion

Consumer-resource relationships could play an important role in ecology, influencing the dynamics of populations and the flow of energy through food webs. Predators can impact prey populations directly through prey consumption (Peckarsky et al. 2008). Consumption of herbivores has a known cost, so it is important to ascertain the number of prey killed when determining the effects of predators on prey population dynamics (Messina & Sorenson 2001). Our results suggest that all instars and adult stages of male and female *N. tenuis* are ravenous feeders and they show strong predatory potential against *T. urticae*.

The number of total mites eaten by male *N. tenuis* (1057) is significantly less than by females (1132) (Tables 1 & 2). Enkegaard et al. (2001) observed that females of *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) are voracious predators of eggs and first instar nymphs of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), as well as eggs of *T. urticae*. Within a 24-h period, one female is capable of consuming more than 80 first instar whiteflies or about 150 whitefly and spider mite eggs. The greater consumption of prey by females is associated with the need to support reproduction, which is a major factor determining their fitness (Pyke et al. 1977, Reznick 1985, Zera & Harshman 2001, Harshman & Zera 2007). Other similar species, like *Macrolophus pygmaeus* (Rambur) females (Hemiptera: Miridae), have telotrophic ovaries and egg production requires a continuous supply of energy throughout adult life with egg development independent of mating (Castañé et al. 2007, Franco et al. 2011). It has been reported that *M. pygmaeus* females consume more prey than males when fed on *Lyriomyza trifolii* (Burgess) (Diptera: Agromyzidae) larvae (Arnó et al. 2003) or *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) eggs (Urbaneja et al. 2009). *M. pygmaeus* also feed on some aphids (Prieto et al. 2016), which is similar to our results.

The present study had predation rates of 10–50%, which are higher than some prior studies. Per day feeding rates ranged from 5.9 to 33.8 mites during first instar to adult for males and 7.9 to 38.4 mites for females in the present study, which are higher than found in other studies on other mirids (Brzezinski 1988, Foglar et al. 1990, Kimsanbaev et al. 1991, Snodgrass 1991, Zhang 1992). In the case of *N. tenuis*, females and males influence in different ways the number of mites consumed depending on the way in which they interacted. It is important, however to highlight that plant architecture and plant surface may affect these interactions. In this context, our results are relevant and highlight the importance of considering predator size in developing models that describe the effect of mirid predation on the age structure of predator colonies.

All of the life stages (first to fifth nymphal instars and male and female adults) of *N. tenuis* were effective predators of *T. urticae*. Among the immature stages, 4^{th} and 5^{th} instars had the highest consumption rates (39.1 for males and 44.4 for females), which were higher than other instars and adults (Tables 1 & 2). Augmentative releases of later instar nymphs of *N. tenuis* against *T. urticae* might therefore assure better control of the mite. Hence, they should be evaluated in the fields for their potential effectiveness when used alone or in combination with other IPM tactics. Similar results have been reported for *Dicyphus amaninii* Wagner (Hemiptera: Miridae), where the release of late-instar nymphs shortly after crop transplant has proved to be a successful strategy for control of thrips, *F. occidentalis*, and whiteflies, *T. vaporariorum,* on cucumber (Castañé et al. 1996).

Nesidiocoris tenuis fed more actively in the presence of adult prey, although all prey individuals were consumed. The predators were probably more aware of the larger size and movements of the adult prey (Prieto et al. 2016). However, *N. tenuis* usually attacked the prey nearest to them. Alomar et al. (2002) found *M. caliginosus* was more abundant in outer crop rows closer to the source of the predator. This mirid species also concentrated on plants with high whitefly densities. Therefore, in the field situation, the density and location of the whiteflies on the crop plants as well as the direction and distance of predator source or reservoir could influence the predation rate of *M. caliginosus* on their prey.

All the active life stages of *N. tenuis* fed well on *T. urticae*. However, later stages were found to be more effective, as the maximum feeding rate was registered by these stages (70–88 mites by male nymph and 86–95 mites by female nymph). The maximum predation by adult stage may be due to the fact this stage is longer compared to other life stages of *N. tenuis*. Similarly, this stage, especially female adults, required more food to perform their routine activities like searching for food and shelter and mate selection.

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