

Article

Behavioral Response of a Specialist Predator, *Stethorus gilvifrons* (Coccinellidae: Coleoptera), to Cope with Web Structures of Four Different Spider Mite Pest Species

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Abstract: The present study aimed to evaluate the web coping and attack behavior of a specialist mite predator, *Stethorus gilvifrons*, against the webbing structures of different spider mite pest species: *Oligonychus afrasiaticus*, *Tetranychus urticae*, *Eutetranychus orientalis* and *O. punicae*. The females of each pest species, in three different treatments, were allowed to construct web structures in the experimental arenas. The predator exhibited three different attack behaviors which were associated with the webbing life types of pest species. Against the *O. afrasiaticus* web, the predator used its palps and mandibles. Against the *T. urticae* web, the predator also made use of its first pair of legs to widen the entry hole it made by cutting. Against the *O. punicae* web, the predator protruded its hypognathous mouthparts to feed on mite individuals by merely entering into the web. Behavioral responses were also found to be associated with webbing life types of pests. Against *O. afrasiaticus* and *O. punicae*, the predator spent much time in web penetration and searching behaviors, respectively. The density-dependent complexity of web structure may cause hindrance to the predator, and it is suggested that adults should be released when a pest colony is growing with low webbing complexity for efficient pest management.



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Keywords: biological control; complicated web; grooming; patrolling; predator web cutting; web invasion; web penetration

1. Introduction

The members of the family Coccinellidae (Insecta: Coleoptera) are very important predators of agricultural insect and mite pests [1,2], varying from specialists to generalists based on their feeding preferences and predation efficiencies [3]. Within the tribe Stethorini Dobzhansky, the genus *Stethorus* Weise includes specialist mite predators [4,5]. The *Stethorus* species are widely distributed throughout the globe in different climatic regions [1,5]. The reason for such distribution is believed to be their intentional introduction to different regions for the biological control of spider mite pests [1,6].

The mites of the family Tetranychidae (Acari: Prostigmata), known as spider mites, are severe pests of economic crops, fruits and vegetables [7]. Their damage is primarily characterized by the construction of webbing structures of varying complexity and densities over the feeding substrate [1,8]. These webbed structures serve as a shelter for the developmental stages and protect against acaricides and predation [8,9]. The characteristics of these webbed structures define the life type of spider mites and are different for different species [8,10–13]. Some spider mite pests produce little webs (LW life type) which only provide protection to eggs, while others make webs of high complexities, irregularities and densities called complicated webs (CW life type) and woven nests (WN life type) [8].

The efficiency and success of a predator in spider mite management largely depend on how it copes with webbing structures [14]. The foraging efficiency of generalist predators decreases with the increase in web complexity [9,15]. Their movement is hindered and

individuals become trapped in the web threads, which may lead to death [16–18]. On the other hand, specialist predators can easily enter and move within complex webs without facing serious hindrances from sticky silken threads through adaptive web coping behaviors [19,20]. In addition, different specialist predators could exhibit different web-cutting behaviors [17].

The spider mite specialist predator *Stethorus gilvifrons* Mulsant is distributed in and adapted to tropical–subtropical climates [1]. Both the adult and larval stages are voracious predators and feed abundantly on different spider mite species [21]. Many different biological and ecological studies have been conducted on *S. gilvifrons* against different spider mite pest species in laboratory conditions [22–27], in greenhouses [28,29] and in open fields [30–33]. However, the behavior of adults when they face webbed structures of spider mites is yet to be explored.

The current research is the first to study the behavioral response of the *S. gilvifrons* when it comes in contact with the web structures of four important spider mite pests, namely date palm mite, *O. afrasiaticus* (McGregor); two-spotted spider mite, *T. urticae* (Koch); citrus brown mite, *Eutetranychus orientalis* (Klein); and avocado brown mite, *O. punicae* (Hirst), under laboratory conditions.

2. Materials and Methods

2.1. Pest Mite Sources

Four different spider mite pest species, with four different webbing life type characteristics, were used in the present study.

- Date palm mite (DPM), *O. afrasiaticus*, belonging to the life type CW–d, collected from infested date palm trees grown in the vicinity of the King Saud University campus;
- Two-spotted spider mite (TSSM), *T. urticae*, belonging to the life type CW–u, collected from infested eggplant plants sown in the greenhouses at King Saud University;
- Citrus brown mite (CBM), *E. orientalis*, belonging to the life type LW, collected from infested citrus plants grown in Education Farm at King Saud University;
- Avocado brown mite (ABM), *O. punicae*, belonging to the life type CW–c, collected from infested *Conocarpus* spp. present in the proximity of the King Saud University campus.

All the collected mite specimens were identified and maintained in the Acarology and Biocontrol Research Laboratory (ABRL) on the suitable host plants until the time of experiment completion. The laboratory conditions were set at 27 ± 3 °C temperature with $50 \pm 5\%$ RH. The identified mite voucher specimens were submitted to the King Saud Museum of Arthropods (KSMA), Acarology section.

2.2. Predator Source

The adults of the predator *S. gilvifrons* were initially collected from *Washingtonia* spp. and identified by experts at KSMA, King Saud University. The population of the predator was monitored at regular intervals. As the pupal stage appeared, that section of plant frond was brought to the ABRL and placed carefully in plastic boxes. The adults emerging from pupae were provided with DPM-infested date fruits and TSSM-infested eggplant leaves to maintain lab stock culture. Later, the adult females from the stock were used in the behavioral experiment.

2.3. Experimental Procedure

For the experimentation, small leaf arenas were made out of suitable host plants for each pest mite species: date palm frond for DPM, eggplant leaf for TSSM, citrus leaf for CBM and *Conocarpus* leaf for ABM. A circular leaf disk (5 cm diameter) was cut in the case of eggplant, citrus and *Conocarpus* plants, while a rectangular frond section (3×5 cm²) was cut in the case of date palm frond. The respective leaf sections were placed over an inverted plastic Petri dish (5 cm diameter) and surrounded with wet cotton to provide moisture to the leaf and restrict mites from leaving the arena. Three treatments for each pest species were set, based on the density of mites released, as 5 (T1), 10 (T2) and 15 (T3) adult

gravid females were released into their respective arenas. Each treatment was replicated eight times. The treatments and the replicates were placed in the laboratory at controlled environmental conditions of 27 ± 3 °C temperature with 50% RH for five days to let the pest mite species construct webbing structures. Each of the pest mite species was allowed to lay eggs, while any immature individuals emerging from eggs were carefully removed without disturbing the web integrity. This was done to keep the number and age of individuals as similar as possible in each treatment and replicate. Furthermore, the eggs were not removed as the place/site of oviposition is among the key characteristics defining the life type of spider mites [8,10,11,34]. After five days, a 12 h starved female *S. gilvifrons* was released in each arena. The arenas were then covered with a plastic Petri dish lid to prevent the predator from flying away.

2.4. Data Recording of *Stethorus Gilvifrons* Response to Pest Mite Webbing

The data, in terms of time spent by female *S. gilvifrons* in various behavioral categories (see Section 2.5.1) for each treatment, were recorded for 1 h right after the predator release. The filming and photography of *S. gilvifrons* response to the webbing structures pest species were performed with an Olympus Microscope Camera (DP72) mounted on an M165 C stereomicroscope (Leica, Wetzlar, Germany). The data recording and imaging were performed according to the protocol mentioned above. After the completion of the first hour of data acquisition, the arena along with the lid was placed back in the laboratory section for 24 h to check the predation efficiency of *S. gilvifrons*. The pest mites consumed during 1 h and after 24 h were also counted in each treatment.

2.5. Data Analysis

2.5.1. Behavioral Categories

There were two major behaviors usually shown by the specialist predators: walking and feeding. These two behaviors can be either inside or outside the web. Additionally, the predator also showed a resting behavior where it stays motionless after satiation or tries to clean the silken web threads off its body parts. In the present study, six behavioral categories were used, following Mirza et al. [18]: (1) patrolling (PG = feeding outside the web), (2) web invasion (WI = walking in the web), (3) web penetration (WP = feeding inside the web after forced entry), (4) searching (S = walking outside of the web), (5) grooming (G = resting inside the web, cleaning body parts while stationary), and (6) resting (R = resting outside of the web, staying motionless). The three first behaviors (PG, WI and WP) were considered predator attack modes. The predator's efficiency was evaluated based on how frequently the various behavioral categories were recorded during 60 min. of observation. Each behavioral category was then individually tested for significant differences among different pest species in each treatment and among different treatments of each pest species.

2.5.2. Statistical Analysis

The time budget data, measured in minutes, for each behavioral category were transformed into ranks with correction for ties. This transformation was done for both treatmentwise and pestwise data analyses. The rank-transformed data were then analyzed by Kruskal–Wallis test with Bonferroni-corrected significance level followed by Dunn's procedure for multiple pairwise mean comparisons. The data for predation were transformed using square-root transformation and analyzed by the tests mentioned above. The data analyses were carried out using XLSTAT (v.2022.5.1.1395, New York, NY, USA).

3. Results

3.1. Observations on Web Coping Behavior of *Stethorus Gilvifrons*

The specialist mite predator *S. gilvifrons* showed different attack and web coping behaviors when it faced the webbing structures constructed by four different spider mite pest species. It was observed that the predator exhibited three different behaviors and one common behavior to forcibly enter the webbing structures. The common behavior was

the use of mandibles and palps to cut the silken web threads. In addition to this, the three different web coping behaviors depended upon the three different webbing structures of three spider mite pest species which are as follows:

(a) In the case of webbing structures of the date palm mite (DPM), *O. afrasiaticus* (life type: CW-d), the predator made use of its large body, covered in posteriorly directed fine hairs, to force its way into the web with forward movements, breaking the integrity of the structure in the process (Figure 1a,b).

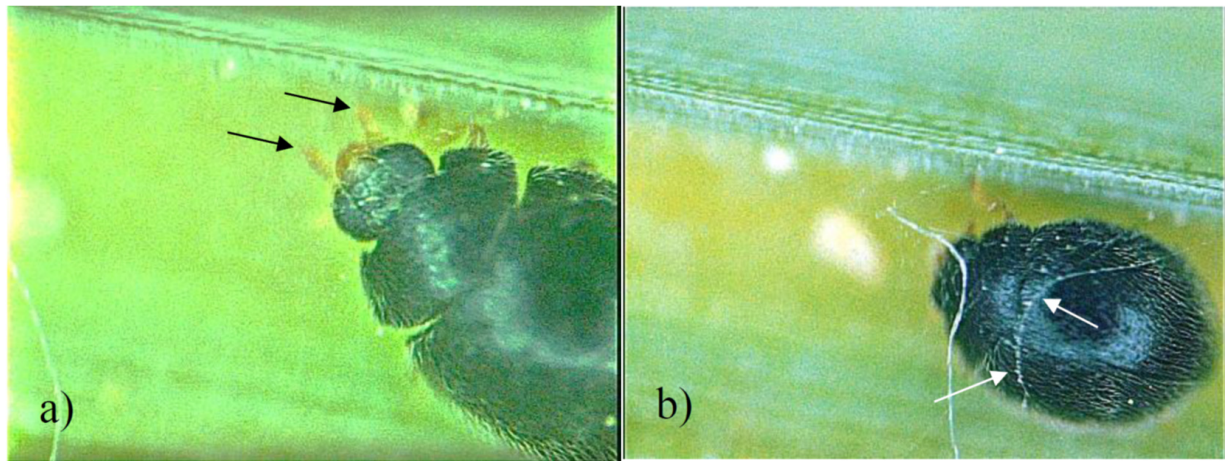


Figure 1. The specialist mite predator *Stethorus gilvifrons* coping with the web of the date palm mite, *Oligonychus afrasiaticus*: (a) using palp and mandibles to cut silk threads; (b) forced entry through forward thrust movement as depicted by web threads over the body. Black arrows represent the use of mandibles, while white arrows represent the web roof being dragged and ruptured by the forced movement.

(b) In the case of webbing structures of the two-spotted spider mite (TSSM), *T. urticae* (life type: CW-u), the predator initially faced difficulty penetrating the webbing structure. To overcome that, the predator first made a small hole on the upper surface of the web by chewing a few silken web threads; then using its first pair of legs and palp, it widened that hole, making it big enough to enter. Later the predator used forward thrust motion, benefiting from the comparatively large body size, to move through the webbing structure (Figure 2).

(c) In the case of the webbing structure of the avocado brown mite (ABM), *O. punicae* (life type: CW-c), the predator only made use of mandibles by protruding its prognathous mouthparts through the web threads and breaking them by chewing, and no web-intrusion behavior was observed (Figure 3).

(d) For the citrus brown mite (CBM), *E. orientalis* (life type: LW), the web threads were only made over the eggs. Although all the pest mites were allowed to lay eggs in their respective arenas, here the predator was found to feed on eggs by first chewing and removing a few constructed web threads and then later consuming the unprotected eggs.

3.2. Attack Behaviors and Predation efficiency of *Stethorus Gilvifrons* against Webbing of Four Spider Mite Pests

The behaviors of patrolling (P), web penetration (WP) and web invasion (WI) were considered as the attack behaviors of the predator. Additionally, the searching (S) behavior was also considered among them for the CBM with the LW life type. The mean patrolling (feeding outside the web) time was higher against TSSM in T3 (14.6 ± 9.3 ; Table 1 and Figure 4), representing almost 24% of all other six behaviors (Figure 5). The mean searching (walking outside the web) time was high against ABM in T1 (27.6 ± 8.1) and CBM in T3 (27.2 ± 3.9), comprising almost 44% of all behaviors against both pest species, separately (Figure 5). The mean time of web invasion (feeding inside the web after forced entry) and

web penetration (walking inside the web) was recorded highest against DPM (Table 1 and Figure 4) followed by that against TSSM, in all treatments (Tables 1 and 2, Figures 4 and 5).

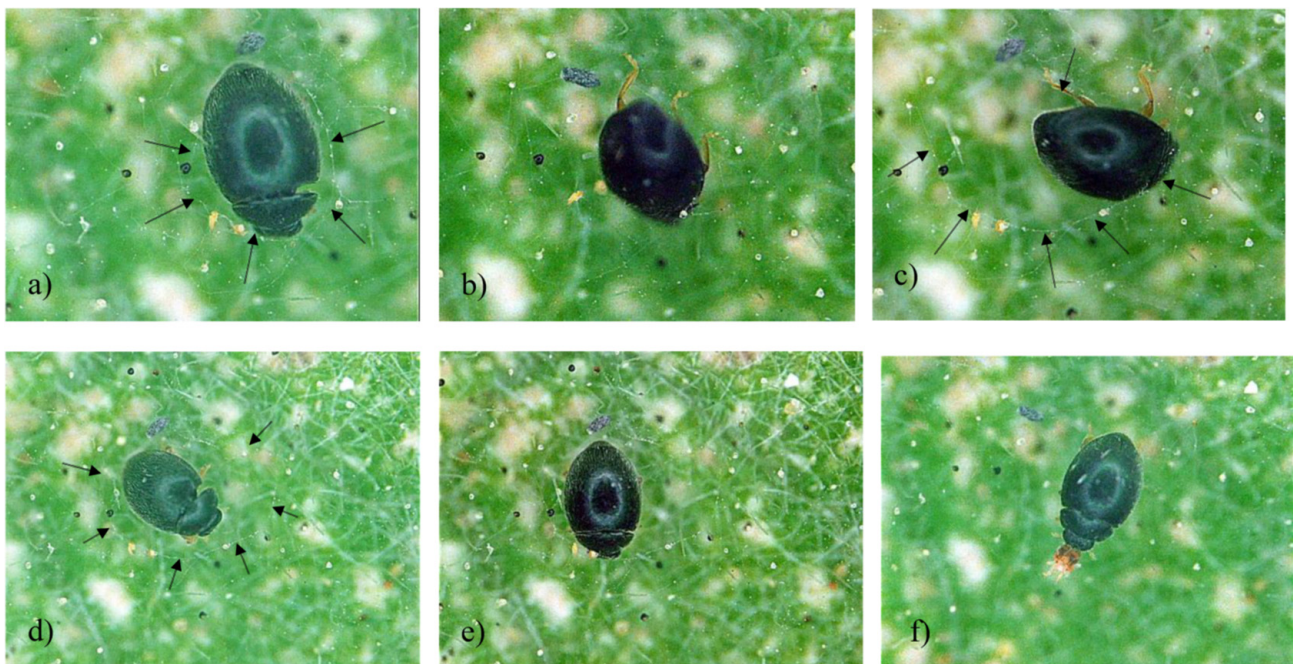


Figure 2. The specialist mite predator *Stethorus gilvifrons* coping with the web of the two-spotted spider mite, *Tetranychus urticae*: (a) predator made entry hole by cutting; (b) predator forcing entry; (c,d) predator using legs and body movement to widen hole; (e) predator widened the hole for easy entry; (f) predator feeding on female mite. Arrows represent the size of the entry hole.

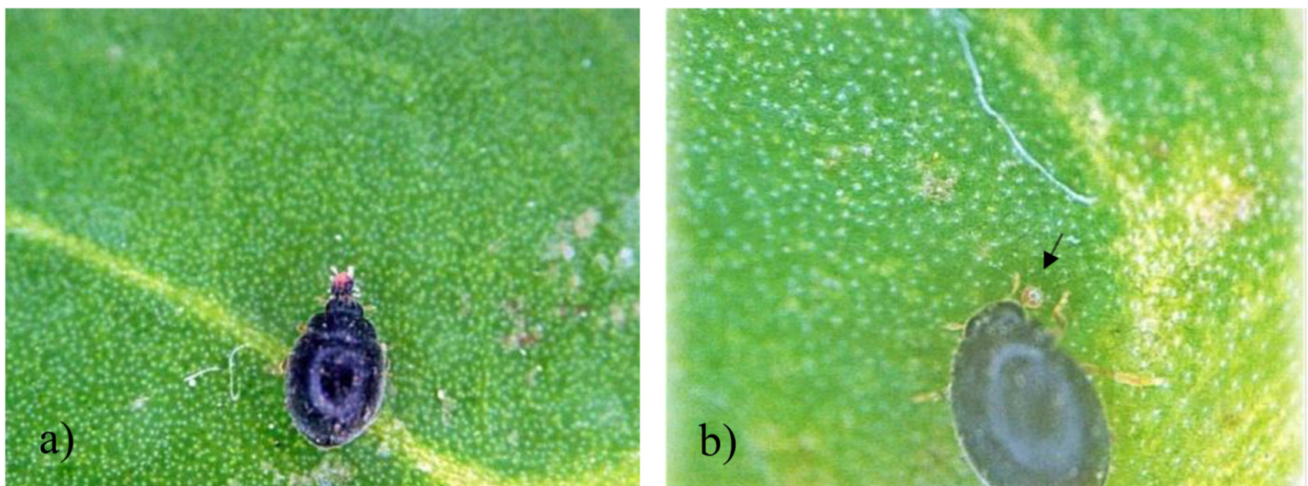


Figure 3. The specialist mite predator *Stethorus gilvifrons* coping with the web of the avocado brown mite, *Oligonychus punicae*: (a) predator feeding on female mite outside the web; (b) predator cutting web threads over the egg. Arrow indicates eggs covered with web threads.

Table 1. Comparison among treatments for the time spent (mean ± std.dev) by the predator *S. gilvifrons* in six behavioral categories against four spider mite pest species (parentheses represent mean ± std.dev of untransformed time measurements).

		Patrolling	Web Invasion	Web Penetration	Searching	Grooming	Resting
DPM	T1	- (0)	16.6 ± 7.3 b (18 ± 7.6)	14.3 ± 8.4 (23.5 ± 6.7)	14.1 ± 7.1 (8.3 ± 5.1)	- (0)	9.4 ± 6.8 (10.1 ± 3.7)
	T2	17.5 ± 6.3 (5.1 ± 4.4)	5.8 ± 3.3 a (8.7 ± 5.5)	11.6 ± 7.7 (20.7 ± 7.2)	12.7 ± 7.4 (8.4 ± 4.5)	- (0)	17.6 ± 5.6 (17.6 ± 8)
	T3	- (0)	15 ± 4 ab (18.6 ± 1.8)	11.5 ± 5.1 (20 ± 3.6)	10.6 ± 6.9 (6.6 ± 4.3)	20.5 ± 2.1 (3 ± 4.5)	10.3 ± 6.5 (11 ± 3.2)
	STAT	DF = 2, p < 0.0001	DF = 2, p = 0.004	DF = 2, p = 0.65	DF = 2, p = 0.6	DF = 2, p < 0.0001	DF = 2, p = 0.03
ABM	T1	-	-	9.9 ± 7.6 (17.7 ± 5.6)	18.7 ± 5.7 b (27.6 ± 8.1)	13.5 ± 9.4 (2.5 ± 1.1)	5 ± 3.2 a (12.3 ± 5.4)
	T2	-	-	11.6 ± 8.2 (17.6 ± 5.9)	7.3 ± 5.9 a (15 ± 6.1)	14.1 ± 6.5 (2.7 ± 1.3)	19.4 ± 4.1 b (24.6 ± 5.2)
	T3	-	-	15.9 ± 3.7 (20 ± 1.5)	11.4 ± 4.1 ab (19.1 ± 2.9)	9.8 ± 3.6 (2 ± 0.7)	13.1 ± 4.1 ab (18.2 ± 2.1)
	STAT	-	-	DF = 2, p = 0.21	DF = 2, p = 0.004	DF = 2, p = 0.41	DF = 2, p = 0.0001
TSSM	T1	13.2 ± 4.3 b (6.3 ± 4)	13.4 ± 6.3 (8.13 ± 5.1)	0 a (0)	12.1 ± 6.4 (13.2 ± 3.3)	4.5 ± 2.3 b (22 ± 3.5)	13.3 ± 7.7 (10.6 ± 7.1)
	T2	0 a (0)	14.8 ± 9.5 (10.3 ± 8.8)	15.1 ± 5.7 b (11.3 ± 4.6)	10.3 ± 6.4 (10.1 ± 6.6)	13 ± 2.8 b (17.7 ± 7.6)	11.8 ± 5.9 (10.5 ± 4.6)
	T3	17.7 ± 7.1 b (14.6 ± 9.3)	9.2 ± 3.1 (2.7 ± 1.6)	15.9 ± 6.9 b (12.2 ± 6.1)	15.1 ± 8.1 (13 ± 8.2)	20 ± 3.1 a (8.6 ± 6)	11.5 ± 7.5 (9.2 ± 6)
	STAT	DF = 2, p = 0.0001	DF = 2, p = 0.23	DF = 2, p = 0.0001	DF = 2, p = 0.4	DF = 2, p < 0.0001	DF = 2, p = 0.77
CBM *	T1	17.5 ± 7.5 b (8.1 ± 5.5)	-	-	10.1 ± 7.6 (22.2 ± 3.9)	-	7.1 ± 3.9 a (29.6 ± 2.5)
	T2	0 a (0)	-	-	9.2 ± 4.9 (22 ± 2)	-	20.1 ± 2.9 b (38 ± 2)
	T3	14.5 ± 2.2 b (1.7 ± 0.7)	-	-	18.1 ± 4.9 (27.2 ± 3.9)	-	10.2 ± 5.7 a (31 ± 4.4)
	STAT	DF = 2, p = 0.0001	-	-	DF = 2, p = 0.02	-	DF = 2, p = 0.001

“-” indicates the behavior was not exhibited by the predator and is considered zero. “*” as the CBM has LW life type, the behavioral categories associated with webbing were not recorded. Multiple pairwise comparisons using Dunn’s procedure alpha = 0.016. Bonferroni-corrected significance level: 0.0053. Means in each column with the same letters are non-significantly different.

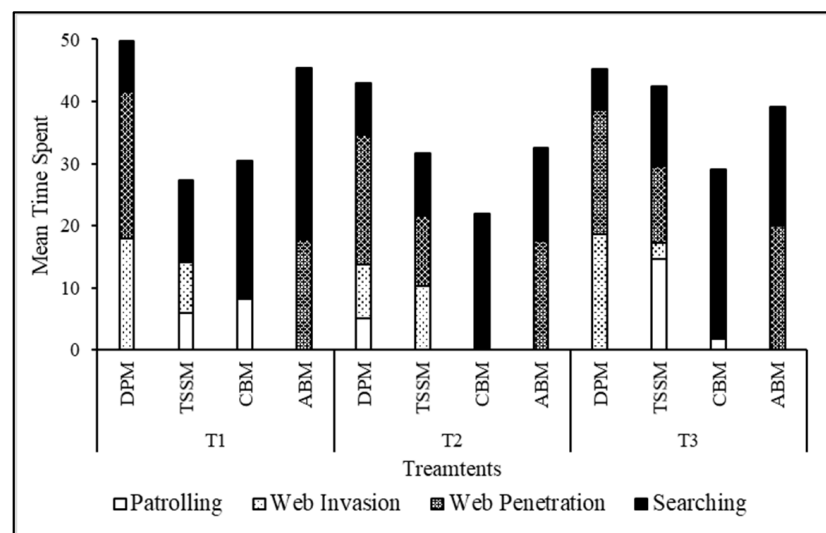


Figure 4. Mean time (min) spent in different attack behaviors by female *Stethorus gilvifrons* against webbing structures of date palm mite (DPM) *O. afasiaticus*, two-spotted spider mite (TSSM) *T. urticae*, citrus brown mite (CBM) *E. orientalis* and avocado brown mite (ABM) *O. punicae*.

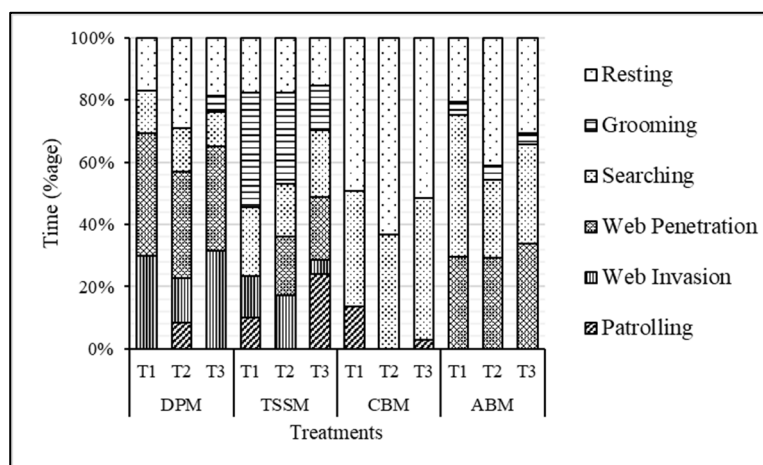


Figure 5. Percent time (min) spent in different behavioral categories by *Stethorus gilvifrons* against webbing of date palm mite (DPM) *O. afrasiaticus*, two-spotted spider mite (TSSM) *T. urticae*, citrus brown mite (CBM) *E. orientalis* and avocado brown mite (ABM) *O. punicae*.

Table 2. Treatmentwise comparison of the time spent (mean ± std.dev) by the predator *S. gilvifrons* in six behavioral categories against four spider mite pest species (parentheses represent mean ± std.dev of untransformed time measurements).

		Patrolling	Web Invasion	Web Penetration	Searching	Grooming	Resting
T1	DPM	0 a (0)	25.5 ± 10.1 b (18 ± 7.6)	26.3 ± 4.7 b (23.5 ± 6)	6.4 ± 5.1 a (8.7 ± 5.1)	0 a (0)	10.1 ± 6.1 a (10.1 ± 3.6)
	ABM	0 a (0)	0 a (0)	22.6 ± 4.2 b (17.7 ± 5.6)	26 ± 6 c (27.6 ± 8.1)	17.5 ± 6.3 ab (2.5 ± 1.1)	14.5 ± 7 ab (12.1 ± 5.4)
	TSSM	22.8 ± 3.9 b (6 ± 3.4)	16.8 ± 10.5 ab (8.1 ± 5.1)	0 a (0)	11.2 ± 4.4 ab (13.2 ± 3.3)	28.5 ± 2.2 b (22 ± 3.5)	13.3 ± 8.8 a (10.6 ± 7.1)
	CBM	26.1 ± 4.9 b (8.1 ± 5.5)	0 a (0)	0 a (0)	22.3 ± 3.9 bc (22.2 ± 3.9)	0 a (0)	27.5 ± 2.5 b (29.6 ± 2.5)
	STAT	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> = 0.001
	T2	DPM	19.5 ± 14.5 b (4.5 ± 4)	21.7 ± 8.9 b (8.7 ± 5.5)	24.5 ± 6.9 b (20.7 ± 7.2)	9.1 ± 5.8 a (8.3 ± 4.5)	0 a (0)
ABM		0 a (0)	0 a (0)	21.4 ± 6.9 b (17.6 ± 6)	17.5 ± 8.1 ab (15 ± 6.2)	20.3 ± 4.9 ab (2.7 ± 1.3)	18.8 ± 3.1 ab (24.6 ± 5.2)
TSSM		0 a (0)	22.2 ± 9.4 b (10.5 ± 8.8)	13.5 ± 7.7 ab (11.3 ± 7.1)	11.8 ± 7.1 b (10.1 ± 6.6)	26.6 ± 6.9 b (17.7 ± 7.6)	6.2 ± 4 a (10.5 ± 4.6)
CBM		0 a (0)	0 a (0)	0 a (0)	27.5 ± 2.7 a (22 ± 2)	0 a (0)	28.3 ± 2.5 b (38 ± 2)
STAT		DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001
T3		DPM	0 a (0)	28.5 ± 2.3 b (18.6 ± 1.8)	24.2 ± 6.7 b (21.2 ± 3.4)	6.3 ± 2.6 a (6.6 ± 3.4)	11.8 ± 12.3 ab (3 ± 4.5)
	ABM	0 a (0)	0 a (0)	22.2 ± 4.3 b (20 ± 1.5)	18.9 ± 4.5 ab (19.1 ± 2.9)	19.5 ± 2.2 b (2 ± 0.7)	20.5 ± 2.5 bc (18.8 ± 2.1)
	TSSM	26.5 ± 5.7 b (14.6 ± 9.4)	19.5 ± 4.6 ab (2.7 ± 1.6)	13.1 ± 9.2 ab (12.2 ± 8.4)	12.1 ± 6.7 a (12.2 ± 7.8)	23.1 ± 10.4 b (8.6 ± 6)	8.5 ± 5.4 a (9.2 ± 6.1)
	CBM	22.5 ± 2.2 b (1.7 ± 0.7)	0 a (0)	0 a (0)	28.5 ± 2.4 b (27.2 ± 3.9)	0 a (0)	28.4 ± 2.5 c (31 ± 4.4)
	STAT	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001	DF = 3, <i>p</i> < 0.0001

Multiple pairwise comparisons using Dunn’s procedure alpha = 0.0125. Bonferroni-corrected significance level: 0.0027. Means in each column with the same letters are non-significantly different.

The predation efficiency of the predator was evaluated based on the number of motile prey individuals it predated during the first hour of observation and after 24 h (Tables 3 and 4). The predator *S. gilvifrons* completely consumed the mite body, as observed for all pest species, including the exoskeleton, leaving no trace behind. Overall, during the first hour, the highest mean numbers of prey consumed were found for DPM (8.3 ± 1.8 in T3) and ABM (6.2 ± 2.6 in T2). After 24 h, significantly different results were found for DPM, CBM and ABM (Tables 3 and 4), and the highest numbers of prey consumed after 24 h were

found for DPM and ABM (Tables 3 and 4). Particularly, in the case of CBM, the data are differential as the predator was observed to feed mostly on the egg stage, both during the first hour of observation and after 24 h.

Table 3. Comparison of prey consumed by the predator *S. gilvifrons* for each pest species in three treatments during 1st hour of observation and after 24 h (parentheses represent mean ± std.dev of untransformed prey number consumed).

	DPM		ABM		TSSM		CBM	
	During 1 h	After 24 h	During 1 h	After 24 h	During 1 h	After 24 h	During 1 h	After 24 h
T1	1.6 ± 0.3 a (2.8 ± 1.1)	2.1 ± 0.8 a (4.4 ± 0.7)	1.9 ± 0.3 (3.8 ± 1.1)	2.2 ± 0.0 a (5 ± 0)	0.5 ± 0.7 (0.7 ± 1.2)	0.8 ± 0.9 (1.5 ± 1.8)	0.7 ± 0.8 (1.1 ± 1.7)	1.3 ± 0.7 ab (2.2 ± 1.6)
T2	2.1 ± 0.3 ab (4.8 ± 1.3)	2.9 ± 0.9 ab (8.8 ± 1.6)	2.4 ± 0.5 (6.2 ± 2.6)	2.9 ± 0.3 ab (8.5 ± 1.7)	0.7 ± 0.6 (1 ± 0.9)	1.7 ± 1.1 (4.2 ± 2.6)	0 (0)	0 a (0)
T3	2.8 ± 0.3 b (8.3 ± 1.8)	3.7 ± 0.2 b (14 ± 1.8)	2 ± 1.1 (5.2 ± 3.8)	2.8 ± 1.1 b (9.2 ± 4.4)	1.1 ± 0.5 (1.4 ± 1.1)	1.8 ± 0.8 (3.5 ± 2.5)	0.7 ± 0.4 (3 ± 1.8)	1.5 ± 0.7 b (3 ± 1.8)
STAT	DF = 2, p = 0.001	DF = 2, p < 0.0001	DF = 2, p = 0.249	DF = 2, p = 0.004	DF = 2, p = 0.197	DF = 2, p = 0.044	DF = 2, p = 0.018	DF = 2, p = 0.001

Multiple pairwise comparisons using Dunn’s procedure alpha = 0.016. Bonferroni-corrected significance level: 0.0053. Means in each column with the same letters are non-significantly different.

Table 4. Comparison of prey consumed by the predator *S. gilvifrons* among four pest species in each treatment during 1st hour of observation and after 24 h (parentheses represent mean ± std.dev of untransformed prey number consumed).

	T1		T2		T3	
	During 1 h	After 24 h	During 1 h	After 24 h	During 1 h	After 24 h
DPM	1.6 ± 0.3 ab (2.8 ± 1.1)	2.1 ± 0.8 ab (4.3 ± 0.7)	2.1 ± 0.3 bc (4.8 ± 1.4)	2.9 ± 0.2 b (8.7 ± 1.5)	2.8 ± 0.3 b (8.3 ± 1.8)	3.7 ± 0.2 b (14 ± 1.8)
ABM	1.9 ± 0.3 b (3.8 ± 1.1)	2.2 ± 0.0 b (5 ± 0)	2.4 ± 0.5 c (6.2 ± 2.6)	2.9 ± 0.3 b (8.5 ± 1.7)	2 ± 1.1 ab (5.2 ± 3.8)	2.8 ± 1.1 ab (9.2 ± 4.4)
TSSM	0.5 ± 0.7 a (0.7 ± 1.1)	0.8 ± 0.9 a (1.5 ± 1.8)	0.7 ± 0.6 ab (1 ± 0.9)	1.7 ± 1.1 ab (4.2 ± 2.6)	1.1 ± 0.7 ab (1.6 ± 0.9)	1.8 ± 0.8 a (4 ± 2.1)
CBM	0.7 ± 0.8 a (1.1 ± 1.7)	1.3 ± 0.7 a (2.2 ± 0.6)	0 a (0)	0 a (0)	0.7 ± 0.4 a (0.7 ± 0.4)	1.5 ± 0.7 a (3 ± 1.8)
STAT	DF = 3, p = 0.001	DF = 3, p = 0.001	DF = 3, p < 0.0001	DF = 3, p < 0.0001	DF = 3, p = 0.001	DF = 3, p < 0.0001

Multiple pairwise comparisons using Dunn’s procedure alpha = 0.0125. Bonferroni-corrected significance level: 0.0021. Means in each column with the same letters are non-significantly different.

3.3. Behavioral Response of *Stethorus Gilvifrons* against Webbing of Four Spider Mite Pests

As the webbing structures (life type) of each spider mite pest species were different, the predator showed differential behavioral responses. Therefore, the results are provided as a comparison of predator behavior among treatments of each species (Table 1) and a comparison of behavior among species in each treatment (Table 2).

3.3.1. Comparison of Predator Behavior among Treatments:

S. gilvifrons vs. *O. afrasiaticus* (DPM): The predator, upon coming in contact with the webbing structure of DPM in all tested treatments, showed significantly different behaviors of patrolling (DF = 2, $p < 0.0001$), web invasion (DF = 2, $p = 0.004$) and resting (DF = 2, $p = 0.03$) (Table 1). The mean time spent exhibiting web penetration behavior was the highest compared to other behaviors of the predator (Table 1, Figures 4 and 5).

S. gilvifrons vs. *O. punicae* (ABM): Against the ABM, significantly different behavioral responses among the treatments were recorded for searching (DF = 2, $p = 0.004$) and resting (DF = 2, $p = 0.001$). However, the longest mean time spent by the predator was in searching (in T1), followed by resting (in T2) and web penetration (in T3) (Table 1). Additionally, the patrolling and web invasion behaviors were not shown by the predator in any of the three treatments (Table 1, Figures 4 and 5).

S. gilvifrons vs. *T. urticae* (TSSM): Against the TSSM, the behaviors of patrolling (DF = 2, $p = 0.0001$), web penetration (DF = 2, $p = 0.0001$) and grooming (DF = 2, $p < 0.0001$) were significantly different among tested treatments, with the longest mean times spent being 14.6 ± 9.3 (T3), 12.2 ± 6.1 (T3) and 22 ± 3.5 (T1), respectively (Table 1). Other than these behaviors, more time was spent searching and resting among all treatments (Table 1).

S. gilvifrons vs. *E. orientalis* (CBM): As little webs (LW life type) were created by this pest species, the behavioral response categories which were associated with webs (web invasion, web penetration and grooming) were not exhibited by the predator (Table 1, Figures 4 and 5). The two behavioral categories of patrolling and resting were significantly different among all the treatments (Table 1). Among these, more time was spent exhibiting the searching and resting behaviors.

3.3.2. Comparison of Behavior among Species

The comparison of time spent by the predator exhibiting different behavioral responses against webbing structures of four spider mite pests is provided in Table 2 and Figure 5. Among the four pest species, all the behavioral response categories were significantly different within a treatment (Table 2). Patrolling (feeding outside the web) was recorded as the least exhibited behavior compared to others (Table 2, Figure 5). The web invasion behavior (walking inside the web) was only measured in high percentages against DPM, followed by TSSM (Figure 5). Similarly, the mean time spent in this behavioral category was highest against DPM (T1: 18 ± 7.6 , T3: 18.6 ± 1.8), followed by TSSM (T2: 10.5 ± 8.8) (Table 2).

Web penetration (feeding inside the web after forced entry) was exhibited more against the web structures of DPM and ABM (Figure 5). The mean time spent for this behavior was highest against DPM and ABM in all treatments (Table 2). Searching (walking outside the web) was highest against CBM and ABM both in the percentage (Figure 5) and mean time spent by the predator (Table 2).

The grooming (staying inside the web, cleaning body) behavior was most exhibited while coping with the web structure of TSSM (Table 2, Figure 5), and the resting (staying outside the web, motionless) behavior was shown against CBM by the female *S. gilvifrons* (Table 2, Figure 5).

4. Discussion

The present research is the first to evaluate and study the attack and web coping behavior of the specialist mite predator *Stethorus gilvifrons* against different webbing structures of date palm mite (DPM), *O. afrasiaticus* (McGregor); two-spotted spider mite (TSSM), *T. urticae* (Koch); citrus brown mite (CBM), *Eutetranychus orientalis* (Klein); and avocado brown mite (ABM), *O. punicae* (Hirst).

The predator *S. gilvifrons* showed differential web coping behaviors against four pest species, each with a specific webbing life type. The predator was observed to use its palps, mandibles and/or front legs. In addition, the forced entry behavior benefiting from the comparatively large body size of *S. gilvifrons* was observed (Figures 1 and 2). Studies on

the web coping behavior of the predators of pest mites are very rare, but they are crucial for the right choice of a biocontrol agent [18]. Previously, a few attempts have been made where the specialist mite predators *Phytoseiulus persimilis* Athias–Henriot and *Neoseiulus californicus* (McGregor) [17,35] and the generalist mite predator *Cydnoseius negevi* (Swirski and Amitai) [18] were evaluated for their responses towards webbing structures of different spider mite pests. It is not unique that the specialist predators show different web-cutting and web-intrusion strategies against web structures of different spider mite pests. For instance, the specialist mite predator *N. californicus* used its palps and chelicerae to cut the silk web threads of TSSM, *T. urticae* [17], while it used the first pair of legs to rupture the walls of the woven nest of *O. perseae* (WN life type) [35]. The differential web coping behavior of *S. gilvifrons* well represented its specialist predation against spider mite pests.

The three species DPM, TSSM and ABM have the complicated web life type (CW). However, they differ in their subtypes of the CW life type [8,10,12,13], and this truly explains the differential behavioral response of the predator (Tables 1 and 2, Figures 1 and 2). The webbing subtypes of DPM and TSSM are very similar to each other but differ in the site of defecation, i.e., on leaf surface vs. scattered on silken web threads, respectively [13]. The purpose of the feces laid on the silk web threads is not only to repel the predators [3,8,36]. These fecal pellets serve as joints or connections, connecting many different web threads at one time, increasing the complexity and irregularity of the webbing structure [37,38]. Hence, the specialist predator *S. gilvifrons* could not easily force its way through the web of TSSM. It had to first cut a hole in the web of TSSM and then widen it by the combined movement of its legs and body (Figure 2). This further explains the high percentage and mean time spent by *S. gilvifrons* in the grooming behavior as it kept cleaning its antennae, palp and legs from sticky silken threads of the web (Tables 1 and 2). The host plant's physical features also hinder predation efficiency [27,39,40]. The presence of leaf hairs was reported to negatively affect the development of *S. gilvifrons* [27]. In the present study, the eggplant, the host plant only used for *T. urticae*, had trichomes. This further explains the low mean number of mites consumed (Tables 3 and 4) and differential web coping behaviors (Figures 4 and 5).

The web structure of ABM is not in a stratified layered form (opposed to that of DPM and TSSM); rather, it is just as high as the height of the mite body [13]. Such a congested space is not suitable for the predator to intrude. The predator just protruded the prognathous mouthparts to feed on ABM individuals dwelling beneath that low-height webbed roof.

The webbing life type of CBM is LW [8], and its individuals only weave a few silken web threads around the eggs and on the surface of the leaves. This is the reason why no web-associated behavior of *S. gilvifrons* was recorded (Tables 1 and 2, Figures 4 and 5). However, the predator showed a preference towards the eggs (data not shown) of CBM rather than feeding on the motile stages. In this specific case, the predator was observed to first chew the few silken threads around the eggs and later consume them completely. It has been reported that the predatory adults of the tribe Stethorini often prefer mite eggs [41,42]. The spider mite eggs are the most nutritious life stage largely because they contain less water [2]. The behavior of *S. gilvifrons* in the present study, i.e., feeding on eggs of only CBM, shows a strong association of the predator with silken web threads and that the eggs were less protected [1] as compared to eggs of DPM, TSSM and ABM. The adults of CBM escaped the predation possibly due to their posture while feeding or resting. They feed/rest on the substrate surface by lying flat on it. This is a unique posture as compared to individuals of ABM, TSSM and ABM (which are usually in standing posture). This has been reported as an escape behavior of a few adult spider mite species [43]. Nonetheless, the stage preference of *S. gilvifrons* needs further investigation. However, this speculation can be tested through previous studies on the life table and functional response of *S. gilvifrons* against CBM [25,27]. Additionally, this predator has been successfully used to suppress DPM populations in date palm orchards [44].

The differences in the predator's attack behaviors (i.e., patrolling, web invasion and web penetration) were found to be associated with different webbing structures (Figure 4). The highest percent and mean time spent in patrolling (feeding outside the web) were observed in T3 of TSSM (Figure 5). Additionally, more time was also spent on web penetration behavior against TSSM coupled with grooming behavior (Tables 1 and 2). The grooming behavior is where the predator takes time to clean the silken web threads off its body parts. The time spent in such behaviors usually reaches a high level either if the predator is not a specialist [18] or if the web is too irregular and dense [45]. Here, the second case is the reason. As discussed earlier, the complexity of the TSSM web structure made it difficult for the predator to intrude. The predator preferred to feed on those TSSM individuals which were wandering out of their webbed home. This further explains the low average number of TSSM prey consumed (Tables 3 and 4). The patrolling and web invasion behaviors against ABM were not recorded in all three treatments (Tables 1 and 2, Figures 4 and 5). The particular reason could be due to the low height of the webbed roof [13]. The behaviors of web invasion and web penetration were highest against DPM, which represents that the predator was least hindered by the web threads and hence predated on more individuals in each treatment during the 1st hour and after 24 h of the experiment (Tables 1 and 2).

5. Conclusions

In the present study, the behavioral responses exhibited by the predator direct us towards a unique association and preference of the predator towards one of the three pest genera. The behavioral data (both observational and analyzed) suggested that *S. gilvifrons* adults preferred to cope with the webbing structures constructed by the pest species of the genus *Oligonychus*. The high mean numbers of mites consumed by the predator during 1st hour and after 24 h were found for DPM and ABM (Tables 3 and 4), both belonging to the genus *Oligonychus*. The literature related to life history traits and functional responses of *S. gilvifrons* also showed this preference towards DPM rather than mites of the genera *Tetranychus* and *Eutetranychus*. Prey preference studies are lacking for *S. gilvifrons* and are needed against different spider mite pest species. This will open future research avenues for *S. gilvifrons* which will definitely improve the application of this specialist mite predator in successful biological control programs. In addition, in light of the findings of the present research, it is suggested that the adults of the predator *S. gilvifrons* should be released at times when the mite population is growing with lower webbing densities.

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