



Plant volatiles mediate orientation and plant preference by the predator *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae)

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Abstract

Control of the red spider mite, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), using the predator *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) is common in integrated pest management programs of vegetable crops such as eggplant (*Solanum melongena*), okra (*Abelmoschus esculents*), and peppers (*Capsicum annum*), but not on tomato (*Lycopersicon esculentum*). A study was conducted to test whether plant volatiles mediate the adult orientation behavior of *C. carnea* on these four crops. The olfactory response of *C. carnea* to volatiles from several vegetable host plants of its prey, *T. ludeni*, was investigated in a Y-tube olfactometer. Volatiles emitted by eggplant, okra, and peppers elicited a positive behavioral response from both *C. carnea* males and females in an olfactometer. Volatiles emitted by mite-infested plants elicited stronger behavioral responses in *C. carnea* males and females than uninfested healthy plants. Odors from mechanically damaged plants and plants with mite damage only attracted the predators. However, odors emanating from mites alone did not evoke any response from *C. carnea*. In contrast, *C. carnea* did not respond to volatiles from uninfested healthy tomato plants or mites with or without damaged plants or mechanically damaged tomato plants. Therefore, the results suggest that odors from eggplant, okra, and peppers are attractive to *C. carnea*, while odors from tomato are not. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: *Chrysoperla carnea*; Neuroptera; Chrysopidae; Eggplant; Okra; Pepper; Tomato; *Tetranychus ludeni*; Orientation; Host finding; Y-tube olfactometer; Plant volatiles

1. Introduction

The red spider mite, *Tetranychus ludeni* Zacher (Acari: Tetranychidae), is one of the most destructive pests of vegetable crops in the sub-tropics (ChannaBasavanna, 1971). This mite feeds on black bean, *Phaseolus vulgaris* L. (Morros and Aponte, 1994), cowpea, *Vigna unguiculata* Savi. (Singh, 1995), mulberry, *Morus alba* L. (Narayanaswamy et al., 1996), sponge gourd, *Luffa acutangula* Roxb. (Koel et al., 1997), *Crotalaria anagyroides* HBK (Sannigrahi and Talukdar, 1998), and can cause substantial losses. Eggplant and okra are often damaged by high densities of mites (Reddy and Baskaran, 1991). Nymphs feed on leaves from inside the web initially, moving out onto the leaf surface as they develop. Foliar feeding causes white stippling and bronzing of the leaves and veins which results in leaf

desiccation and leaf loss. Sometimes, leaves become coppery and then brittle as a result of feeding by *T. ludeni* (Ansari and Pawar, 1992). Adults often spin silken threads over leaves, forming profuse webbing, which provides a suitable microhabitat for the replenishment of the colony (Sumangala and Haq, 1994). Their rapid developmental rate and high reproductive potential enable them to reach damaging population levels very rapidly under growing conditions. *T. ludeni* populations which occur throughout the year in the sub-tropics become higher during April and reach peak numbers from May to July (Puttaswamy and ChannaBasavanna, 1980). However, population increases are associated with periods of low rainfall and relative humidity and high temperatures.

Green lacewings are considered to be one of the most effective generalist predators (New, 1975). They feed on Lepidoptera eggs and young larvae, aphids, spider mites, scales, psylla, mealybugs, whiteflies, thrips, leafhoppers, and other soft-bodied prey (Canard et al.,

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1984; New, 1988). Lacewings are highly predacious and cannibalistic as larvae (Nordlund, 1993). Larvae insert their mandibles into their prey, inject digestive enzymes, and suck out body fluids (Olkowski et al., 1991) while adults mostly feed on nectar, pollen, and honeydew and some are predatory (Coppel and Mertins, 1977). Past research has demonstrated that lacewings are effective predators of aphids on red peppers (Hassan, 1976), mites on apples (Miszczak and Niemczyk, 1978), and leaf hoppers on grapes (Daane, 1993). Effective control can be achieved by releasing relatively large numbers of lacewing larvae. For example, Daane and Yokata (1997) used between one and sixteen lacewings per plant for control.

In previous studies, we used augmentative releases of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) as one of the viable components of integrated pest management (IPM) of *T. ludeni* on eggplant (Reddy, 2001). Effective control of spider mites using *C. carnea* has been reported several times (Lo et al., 1989; Pal et al., 1989; Sharanabasava and Manjunatha, 1998a,b). However, a previous attempt to control *T. ludeni* in tomato with *C. carnea* releases failed. Moreover, only a low-level predator population was observed in the tomato planting. In contrast, surrounding cabbage, pepper, eggplant, and okra fields had comparatively high predator densities. Therefore, this study was conducted to determine if *C. carnea* use plant odors directly from the host plants to eventually locate its prey. The results of orientational responses of *C. carnea* in a Y-tube olfactometer toward volatiles from several plants that are major *T. ludeni* hosts are presented.

2. Materials and methods

2.1. Predators

Chrysoperla carnea, collected from a cabbage field, were reared on larvae of *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) at $28 \pm 2^\circ\text{C}$, 60–65% RH, and 16:8 (L:D) h as described in a previous study (Reddy et al., 2002). Eggs were placed individually in plastic cups (6 cm long \times 3 cm ID) and after hatching, larvae were fed with young *P. xylostella* larvae, which were fed on cabbage leaves. Upon emergence, newly emerged *C. carnea* adults were collected daily, fed with honey: fructose:protinex:water at 1:1:1:1 by volume, and transferred to acrylic cages (28 cm diameter, 15 cm high) for oviposition. *C. carnea* males and females (3–6 d old) were used for tests. Although adult male and female *C. carnea* are similar in overall size and appearance, they are sexed by examination of the ventral surface of the abdominal tip. Males have a small rounded sac-like structure, flanked by two small projections while females have an oval area bounding a longitudinal slit.

2.2. Culturing of mites

An isogenic culture of *T. ludeni* was maintained in the greenhouse at 14:10 (L:D) h, $30 \pm 2^\circ\text{C}$, and 60% RH. Mites were reared on eggplant, okra, pepper, and tomato plants. The cultures were initiated with one plant of each crop per pot inside a wire mesh cage with one variety per cage. One male and female mites were introduced onto a 15-d-old plant of each variety. All the plants were covered with fine mesh netting to prevent mite emigration. When the plant was badly damaged by mite injury, fresh plants were added and the damaged plants were removed after the mites had migrated to the healthy one. Approximately 20 generations were completed before we used these mites for experiments. For all investigations, mites were obtained from the respective cultures.

2.3. Plants

The plant species used as odor sources in the experiments were eggplant, (*Solanum melongena* L. cv. Pusa kranthi, Solanaceae), okra (*Abelmoschus esculents* L., cv. Pusa savani, Malvaceae), peppers (*Capsicum annum* L., cv. Byadagi, Solanaceae), and tomato (*Lycopersicon esculentum* Mill., cv. Megha or L-15, Solanaceae). The plants were raised in a nursery in the greenhouse ($25\text{--}30^\circ\text{C}$, 60–80% RH, 16:8 (L:D) h) and each transplanted individually to a pot filled with a mixture of 2 kg sand, 2 kg farm yard manure (FYM) and 2 kg loamy clay soil (pH 6.5 available P_2O_5 5.3 mg/100 g, K_2O 7.5 mg/100 g); 1.5 g nitrogen was applied as NH_4NO_3 , 2.0 g phosphorous as Ca (H_2PO_4), 3.0 g potassium as KCL, and K_2SO_4 (50/50). All fertilizer compounds were mixed in the dry soil as solutions except for P, which was added as pure salt. Moisture content was adjusted at about 60% of capacity and held at this level by irrigating during the experiment. The plants were grown under natural light and temperature conditions. All the plants were covered with fine mesh netting to protect them from other possible insect attack. For the different experiments, the plants chosen were at a stage (2–2.5 months old) when high *T. ludeni* infestations generally occur.

2.4. Bioassay

A Y-tube olfactometer, which has been described previously by Reddy et al. (2002), was used to investigate the orientational response of the predator toward plant volatiles. The method of testing the whole live plants was according to Ngi-song et al. (1996). The test materials were placed in two plastic chambers (40 \times 40 \times 140 cm), which was quite enough to accommodate whole live plants (2–2.5 months old). One of the square ends of the chamber was left open. For the system to be air tight, the open end of each box was placed

over the test materials, which stood in water held in a plastic basin. The two chambers were connected to the arms of the Y-tubing from the top of the chambers. One vacuum pump drew and pushed air through the closed system. By using air pressure, an airflow was generated through activated charcoal and drawn into the Y-shaped glass tubing of the olfactometer made of transparent Plexiglas (3 cm, ID; stem 10 cm, arms 8 cm; stem–arms angle 130°). TiCl₄ smoke was used to verify the airflow through the olfactometer. The airflow through each olfactometer arm was 1.2 l/min, which was observed with a flowmeter. All experiments were conducted during the day at ambient conditions at 28 ± 2 °C and 60–70% RH because lacewings appear active almost all of the time, although their peak activity period is for the first 2 h after dark. A single adult of *C. carnea* was introduced into the Y-olfactometer at the entrance of the stem, with maximum observation duration of 10 min per predator and the responses were considered positive when the insects reached at least 4 cm along the arm connected to the test chambers. Predators that did not make a choice within 10 min time period were excluded from the data analysis. Data were collected with the behavior-recording program of the software “The Observer” (Noldus Information Technology, Wageningen, The Netherlands). Insects were used only once and discarded. Thirty individuals of each sex were tested with each test material. After every three runs, the olfactometer and the test chambers were thoroughly washed in soap and water, rinsed in 70% ethanol, and dried in an oven at 120 °C. After five runs the apparatus was rotated 180° to exclude directional bias. The mean percentage of time rested by the predator in each arm of the olfactometer was determined and values submitted to statistical analysis using the binomial sign-test (SPSS) and differences between means were tested using the LSD method.

2.5. Response to volatiles

Live healthy plants. Uninfested live healthy potted plants (2–2.5 months old) of eggplant, okra, peppers,

and tomato were used in the experiments. The plants were tested singly in the chambers connected to the olfactometer.

Infested plants. Potted eggplant, okra, peppers, and tomato plants were inoculated with 300 spider mites. On each plant, three leaves were selected randomly, one per top, middle, and bottom of the plant. One hundred mites were introduced at each of these three places per plant. After inoculation, the plants were covered with polythene cages to prevent mite emigration. Mites were allowed to feed for 48 h, and tests were conducted on the third day after inoculation.

Mite-damaged plants. Similarly potted plants of the above mentioned were infested with 300 spider mites separately and the mites were removed using a camel hair brush after feeding for 48 h and tests were carried out on the third day in a olfactometer.

Mechanically damaged plants. Plants were artificially damaged by scraping three leaves (1 cm²) on the plant, each one from bottom, middle, and top of the plant with a fine blade, 1–2 h prior to the bioassay.

Spider mites alone. This test was conducted to determine whether mites alone could produce kairomones that may attract adult *C. carnea*. Mite loads of 100, 300, 900, and 1200 mites were introduced into a 250 ml glass container, which was connected to one arm of an olfactometer, while the other arm without prey served as blank.

3. Results

Overall results showed that *C. carnea* responded significantly differently to the various plant volatiles tested in the olfactometer ($F = 6.42$, $df = 2$, $P = 0.01$). Both sexes were significantly attracted to odors from eggplant, okra, and peppers with feeding mites (damage with mites) than uninfested healthy plants (Table 1). However, tomato volatiles elicited no behavioral responses from *C. carnea* males or females (LSD, $P \leq 0.05$). *C. carnea* males exhibited a significant preference for eggplant over okra ($F = 10.56$, $df = 5$,

Table 1
Response of *Chrysoperla carnea* to intact healthy plants vs. plants with feeding mites (with mites and damage) in a Y-tube olfactometer

Plant	Mean percentage of time (% ± SD) spent in arm ^a							
	Male				Female			
	Uninfested healthy plants	<i>N</i>	Mite infested plants	<i>N</i>	Uninfested healthy plants	<i>N</i>	Mite infested plants	<i>N</i>
Eggplant	26.5 ± 18.7	5	54.0 ± 14.3a***	25a ^b	28.2 ± 16.6	4	56.0 ± 17.5a***	26a ^b
Okra	24.7 ± 12.3	7	38.2 ± 12.6b**	20b ^b	24.8 ± 13.3	6	48.4 ± 18.6a***	24a ^b
Pepper	20.4 ± 13.7	6	36.3 ± 12.2b**	17b ^b	22.3 ± 11.5	8	39.3 ± 13.8b**	18b ^b
Tomato	24.6 ± 11.6	5	20.0 ± 10.8c	7c	22.3 ± 12.7	6	26.7 ± 14.5c	8c

^a Asterisks indicate significant differences within columns (Binomial sign-test), 30 replicates: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Figures in a column with different letters are significantly different at $P \leq 0.05$ by LSD. *N* indicates the number of *C. carnea* adults responding in the respective arms.

^b Significant differences between within columns (Binomial sign-test, $P \leq 0.05$).

Table 2

Response of *Chrysoperla carnea* to intact healthy plants vs. plants with mite feeding damage (mites removed after feeding; then tested) in a Y-tube olfactometer

Plant	Mean percentage of time (% ± SD) spent in arm ^a							
	Male				Female			
	Uninfested healthy plants	N	Plants with mite feeding damage	N	Uninfested healthy plants	N	Plants with mite feeding damage	N
Eggplant	22.4 ± 16.2	6	46.2 ± 18.6a***	24a ^b	24.3 ± 13.3	5	48.0 ± 18.3a***	25a ^b
Okra	20.2 ± 12.7	7	35.0 ± 12.4b**	18b ^b	19.6 ± 11.7	6	44.2 ± 13.4a***	24a ^b
Pepper	27.8 ± 16.3	5	34.4 ± 13.7b**	17b ^b	20.8 ± 16.6	7	36.7 ± 10.2b**	17b ^b
Tomato	22.3 ± 13.2	7	21.5 ± 10.6c	6c	21.2 ± 12.8	5	23.4 ± 11.7c	6c

^a Asterisks indicate significant differences within columns (Binomial sign-test), 30 replicates: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Figures in a column with different letters are significantly different at $P \leq 0.05$ by LSD. N indicates the number of *C. carnea* adults responding in the respective arms.

^b Significant differences between within columns (Binomial sign-test, $P \leq 0.05$).

Table 3

Response of *Chrysoperla carnea* to intact healthy plants vs. plants with mechanically damaged in a Y-tube olfactometer

Plant	Mean percentage of time (% ± SD) spent in arm ^a							
	Male				Female			
	Uninfested healthy plants	N	Plants with mechanical damage	N	Uninfested healthy plants	N	Plants with mechanical damage	N
Eggplant	24.3 ± 16.3	6	45.0 ± 17.9a***	24a ^b	25.8 ± 11.5	5	46.3 ± 16.4a***	25a ^b
Okra	22.5 ± 12.6	8	33.7 ± 15.2b**	19b ^b	22.5 ± 13.2	6	42.5 ± 18.7a***	24a ^b
Pepper	18.8 ± 18.4	5	34.5 ± 13.8b**	17b ^b	20.4 ± 17.8	5	33.4 ± 14.2b**	18b ^b
Tomato	23.4 ± 15.2	7	22.3 ± 14.6c	6c	21.1 ± 15.2	8	24.5 ± 12.6c	6c

^a Asterisks indicate significant differences within columns (Binomial sign-test), 30 replicates: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Figures in a column with different letters are significantly different at $P \leq 0.05$ by LSD. N indicates the number of *C. carnea* adults responding in the respective arms.

^b Significant differences between within columns (Binomial sign-test, $P \leq 0.05$).

$P = 0.001$) and pepper while eggplant and okra were preferred over pepper by females ($F = 6.16$, $df = 5$, $P = 0.0127$).

Adults of *C. carnea* spent significantly more time in the olfactometer assay with plants with odors from mite feeding damage than with odors from undamaged plants even after mites were removed ($F = 12.57$, $df = 2$, $P = 0.0149$) (Table 2). Here again, the tomato plants with mite feeding damage did not significantly attract *C. carnea* although plants were infested at the same mite density (300 mites were fed for 48 h). The preference of both sexes of *C. carnea* for attractant plants (eggplant, okra, and peppers) after removal of mites was consistent as above.

When mechanically damaged plants were offered in dual choice tests, both sexes of *C. carnea* preferred odors from damaged plants over odors from intact healthy plants ($F = 14.12$, $df = 5$, $P = 0.0018$) (Table 3). The predator reacted most strongly to odors from damaged eggplant and somewhat less to odors from mechanically damaged okra and pepper plants. Odors emanating from mechanically damaged tomato plants did not induce a significant response.

Mites alone did not attract either sex of *C. carnea* in an olfactometer even at the highest mite load of 1200.

There was no significant difference in predator responses among the mite load densities used in the test arm ($F = 0.13$, $df = 1$, $P = 0.4378$) (Fig. 1). Although females spent numerically longer times than the males in all the tests, significant differences could not be found ($P \geq 0.05$).

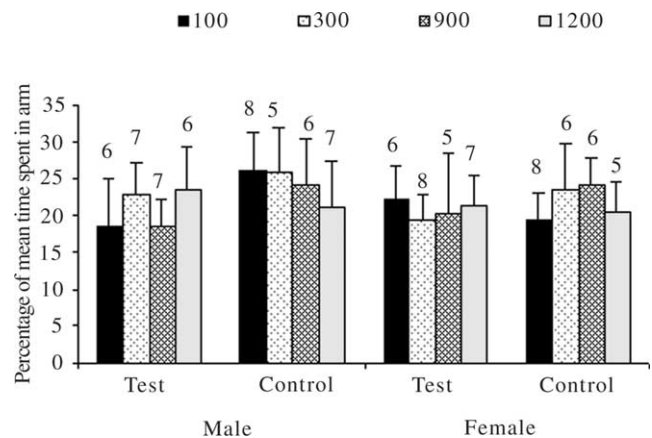


Fig. 1. Response (mean ± SD) of *Chrysoperla carnea* to different spider mite densities (100, 300, 900, and 1200) in an olfactometer. Mean of 30 replications. Bars with the number indicate *C. carnea* adults responding in the respective arms.

4. Discussion

Predators have been known to respond to plant volatiles (Whitman, 1988). Several studies have demonstrated that parasitoids are attracted not only to the volatiles from pest-infested host plants, but also to intact host and nonhost plants (Takabayashi et al., 1991). However, such studies on predators are scarce. Our results indicated that the volatiles from eggplant, okra, and peppers attracted *C. carnea* adults in an olfactometer. To our knowledge, there are few reports on the response of *C. carnea* to different plant volatiles. Both sexes of the predator highly preferred the odors emanating from eggplant followed by okra and peppers. These results are in agreement with Hassan (1978) who observed total elimination of aphid populations *Myzus persicae* (Sulzer) on eggplant by *C. carnea* while HaAbd-Rabou (1999) reported that *C. carnea* are the most abundant potential predator of the whitefly *Bemisia tabaci* (Gennadius) on eggplant. In the present study, *C. carnea* preferred okra and peppers next to the eggplant. Although, Ames and MacLeod (1990) identified several volatile components in okra, no attempt has been made thus far to study the responses of insect pests or natural enemies to these volatiles. However, Zaki et al. (1999) reported that double releases of *C. carnea* (1:5 predator:aphids) in plots grown with okra achieved 100% reduction in *Aphis gossypii* Glover after 12 d. Srivastava et al. (1996) achieved good control of pests in peppers, barley, cotton, and cowpeas by releasing *C. carnea* uniformly by a *Chrysoperla* larval applicator.

In the present study, odors emanating from tomato plants did not attract *C. carnea* in the olfactometer. Tomato plants are known to produce many volatile chemicals that elicit searching behavior in parasitoids (Nordlund et al., 1985). However, there are few reports on the responses of predators to tomato volatiles. Takabayashi and Dicke (1992) observed the positive responses of the predatory mite *Phytoseiulus persimilis* Athias—Henriot (Acarina: Phytoseiidae) to volatiles from tomato and Lima bean leaves in a Y-tube olfactometer. Takabayashi et al. (2000) reported that tomato leaves attracted *P. persimilis* when plants were slightly infested with the red strain, or moderately or heavily infested with the green strain of the mite, *Tetranychus urticae* (Koch). In contrast, neither leaves that were slightly infested with green-strain mites, nor leaves that were moderately or heavily infested with the red strain attracted the predators. Andersson et al. (1980) identified 12 volatiles and reported that the chemical characteristics of commercial tomato cultivars are very homogeneous. Nordlund et al. (1984) observed parasitization by *Trichogramma* sp. was higher on tomato plants compared to adjacent corn or beans. Further, volatile synomones in tomato plants stimulate search behavior in *Trichogramma* (Nordlund et al., 1985). Ei-

genbrode et al. (1995) indicated in their greenhouse studies that *C. carnea* always significantly reduced the survival of *P. xylostella* larvae on glossy cabbage plants, but never on normal-wax plants, and the greater effectiveness of predators on glossy plants could be due to the reported improved mobility of these insects on glossy leaf surfaces.

However, there are few reports on the responses of *C. carnea* to other plants. This predator did not prove very successful for control of aphids on cucumbers, particularly in summer months (Beglyarov and Ushcheckov, 1974). Ballal and Singh (1999) reported that *C. carnea* males had a significantly higher orientation preference for sunflower and females for both sunflower and cotton, while pigeon pea was least preferred in a wind tunnel. Flint et al. (1979) reported that damaged cotton plants released the terpenoid β caryophyllene, which attracts *C. carnea*. Among the green leaf volatiles from cabbage (*Brassica oleracea* subsp. *capitata*), only Z3-6:Ac elicited strong responses in both sexes of *C. carnea* in an olfactometer and the attraction was higher when the volatile was mixed with the pheromone blend in 1:1 ratios (Reddy et al., 2002). In the present study, the strong preference exhibited by *C. carnea* to eggplant, okra, and pepper could be due to host plant kairomones or visual cues. It would be interesting to know the specific factors responsible for this strong response of *C. carnea* toward damaged plants.

The responses of predators vary largely with host-plant species (Dicke and Sabelis, 1988; Takabayashi and Dicke, 1992). In the present study, it was observed that *C. carnea* responded significantly more to the damaged plants than to intact healthy plants. These results agree with Dicke and Sabelis (1988) who reported that predatory mites are only attracted towards spider mite-damaged pear leaves. Further, both sexes of predators did not respond to only spider mites in the olfactometer. These findings are not in agreement with Sengonca et al. (1995) who reported that the first and third instar larvae of *C. carnea* responded significantly to the kairomones of the prey species tested including the spider mite *T. urticae*. Thus damage by phytophagous mites induces plants to produce volatiles that attract predators.

In conclusion, the present findings contribute new data pertaining to the attraction of *C. carnea* to chemical cues associated with the plants. Although in the present study, we used *C. carnea* adults, which are not predatory, but there would be clear reproductive advantage for both males and females. Therefore, these results provide support for releasing *C. carnea* to control spider mites on eggplant, okra, and peppers. The release of *C. carnea* in tomato fields may not be successful. However, further studies are required on the impact of trichomes and trichome exudates of tomato plants on *C. carnea* to understand the interaction between host plant-predator. Elsey (1974) reported that *C. carnea* were able to search

for their prey at a much greater speed on the leaves of cotton than those of tobacco because the movements of the larvae were seriously hampered by the glandular trichomes on tobacco. The trichomes present on cotton were not glandular and were sufficiently sparse that they did not impede the larvae.

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