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Toxoptera citricida (Kirkaldy) (Homoptera:Aphididae) Aqueous Extracts Elicit Enhanced Oviposition Response by Its Parasitoid *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae)

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Abstract : The Brown citrus aphid, *Toxoptera citricida* (Kirkaldy) is a serious pest of *Citrus* spp. as it is the most efficient vector of Citrus Tristeza Virus (CTV) which causes significant reduction in citrus production worldwide. Management of *T. citricida* in citrus orchards is usually by chemical and to a lesser extent biological control. The parasitoid *Lysiphlebus testaceipes* (Cresson) is a fairly efficient natural enemy of *T. citricida* however levels of parasitism vary throughout its range. Kairomones provide one avenue to increase levels of parasitism but this has not been thoroughly investigated for this pest-parasitoid complex. *Lysiphlebus testaceipes* was exposed to various concentrations of *T. citricida* aqueous extract on citrus leaves (host patch) and its leaf and host arrival times, first escape time, number of antennal and oviposition contacts and total time of contact with the host patch determined. Female *L. testaceipes* responded positively in all cases to aqueous extract of host sprayed on a host-infested leaf. Mean leaf arrival time by *L testaceipes* increased with concentration of host extracts. *L. testaceipes* host arrival time was less at all concentrations compared to the control and was fastest at 50 aphids/ mL H_2O. First escape time at all host extract concentrations was significantly different from that of the control and there was a very high positive correlation between mean number of antennal contacts with the host and extract concentration. Likewise, the mean number of oviposition pricks gradually increased with increasing extract concentration and more hosts were ultimately oviposited in at higher host extract concentrations. The mean total time spent by *L. testaceipes* in contact with *T. citricida* increased approximately linearly ($y = 3.05x + 772.16$, $R^2 = 0.78$) with increasing host extract concentrations and was significantly different from the control.

Key words: Host arrival, antennal contact, *Lysiphlebus testaceipes,* brown citrus aphid, kairomones

Introduction

Aphids are an extremely successful group of insects which exist throughout the world, with the greatest number of species in temperate regions (Dixon, 1997; van Emden and Harrington, 2017). Many species are agricultural pests and tree dwelling aphids can severely retard the growth of their host plants by curling leaves and stunting the growth of young stems. The brown citrus aphid *Toxoptera citricida* (Kirkaldy) (Homoptera:Aphididae) is important because it feeds on a wide range of *Citrus* spp., can develop enormous populations in a short time and is the most efficient vector of Citrus Tristeza Virus (CTV). One of the most devastating citrus crop losses as a result of CTV was reported in Brazil and Argentina where approximately 16 million citrus trees on sour orange rootstock (*Citrus aurantifolia*) were killed (Rocha-Peña *et al.,*1995; Halbert and Brown, 1996). The movement of the brown citrus aphid from Venezuela in 1989 continued, eventually appearing in the Caribbean islands of Trinidad in 1985, Guadeloupe and Martinique in 1991; Cuba, Dominican Republic, Puerto Rico and St. Lucia in 1992; Antigua and Barbuda, Jamaica, St. Kitts and Nevis, St. Vincent and the Grenadines in 1993 and Belize in 1996 (Yokomi *et al.,* 1994; CABI, 2020).

Aphids are attacked by a wide variety of predators, pathogens and parasitoids including several species of parasitoid wasps, ladybird beetles, lacewings and entomopathogenic fungi. These natural enemies assist in preventing aphid populations from increasing to economically damaging levels. One such aphid parasitoid, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) has shown potential for regulating the population of its host, the brown citrus aphid *T. citricida* in citrus orchards (Yokomi and Tang, 1996; Balfour and Khan, 2012). Interest in using biological control in such situations has increased in recent years due to reduced adverse effects on non-target organisms and the environment in general compared to the use of synthetic insecticides. Parasitoid behaviour appears to be strongly influenced by semiochemicals which can be of great benefit in designing insect pest management programmes that are more effective and environmentally friendly (Weseloh, 1981; Grasswitz and Paine, 1992). The current study was designed to determine what effect host aphid (*T. citricida)* extract has on host location and oviposition behaviour of its parasitoid *L. testaceipes*.

Materials and Methods

Insect collection and rearing

T. citricida were collected from the University of the West Indies Field Station, Mount Hope (10.6375^0 N, 61.4275^0 W) and the Ministry of Agriculture Central Experimental Research Station, Centeno (10.5973^0N, 61.3166^0W), Trinidad. The aphids were taken to the laboratory and placed on young shoots of potted 4-month old sweet orange (*Citrus sinensis)* plants. The experimental procedure was similar to that of Srivastava and Singh (1988). First and 2nd instar *T. citricida* nymphs were left to moult and the fourth instar to reproduce.

Lysiphlebus testaceipes mummified *T. citricida* aphids on citrus leaves were collected from the orchards mentioned above. Mummies were carefully removed from the leaves and placed in clear 20mL plastic containers to ensure that emerging parasitoids were not exposed to any aphids prior to being used in bioassays. The covers of the containers were fitted with very fine mesh cloth (250mesh/cm^2) to allow for aeration and prevent the emerged wasps from escaping. Adult wasps were fed with honey and 1-day old mated females were used in bioassays. Females were easily identified by their size, being larger than the males and by their abdomen, which was larger and taped compared to males.

Bioassays

A young *C. sinensis* leaf approximately 2 x 1.3 cm was placed with its upper surface down on filter paper moistened with distilled water in a 15cm petri dish. Fifty 3rd instar *T. citricida* aphids were placed on the leaf and allowed to settle. The leaf was sprayed with 0.5mL of a host extract comprising of either: 5, 10, 25, 50, 100 or 200 homogenized, 3rd instar *T. citricida* nymphs/1mL of distilled water. This host extract was used immediately after preparation to reduce the possibility of deterioration. The leaf was left to dry for 5 minutes. One mated 1-day old female wasp was introduced into the covered petri dish-aphid-leaf arrangement (= host patch) and her behaviour observed for 30 minutes. Ten replicates of each experiment were performed for each concentration. The following behaviour patterns of the parasitoid were recorded: the leaf arrival time, this is the period between introduction and first contact with the leaf; host arrival time, this is the period between introduction and first contact with the host; first escape time, this is the time between introduction and first escape from leaf; number of antennal contacts, number of oviposition contacts, and the total time of contact with the host patch (leaf).

Statistical analysis

Data were subjected to one way ANOVA and Tukey-Kramer *post hoc* test if significance (P=0.05) was found using Minitab® 18 software. Mean values, standard errors and 95% confidence intervals were calculated and presented. Scatter plots of the data were also done and best fit models based on examination of residual plots and adjusted R^2 values were generated.

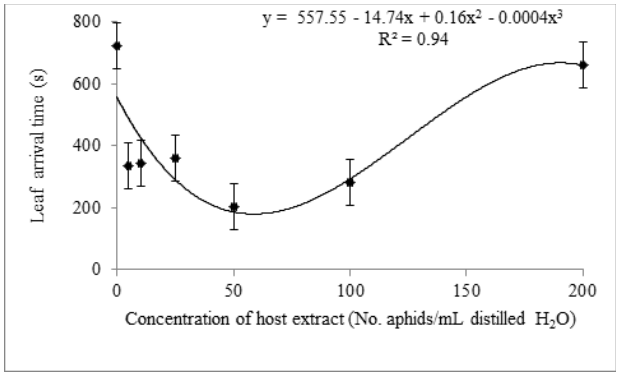

Fig. 1. Leaf arrival time by *Lysiphlebus testaceipes* exposed to different concentrations of host Extracts.

Results and Discussion

The period between introduction of the parasitoid and its' first contact with the host patch that is, the leaf arrival time increased curvilinearly (Fig. 1). There was a significant ($F_{5, 59}$ = 4.42, P<0.05) relationship between the mean leaf (=host patch) arrival time by *L testaceipes* and increasing concentration of host extracts. *L. testaceipes* took the shortest time (P<0.05) to arrive at the host patch when exposed to 50 aphids/mL H_2O compared to either the control or host extract of 200 aphids/mL H_2O (Table 1). This relationship was best described by the model $y = 557.55 - 14.74x + 0.16x^2 - 0.0004x^3$ ($R^2 = 0.94$) (Fig. 1).

Leaf arrival time increased at host extract concentrations <50 aphids/mL H_2O and >100 aphids/mL H_2O indicating that *L. testaceipes* was taking a longer time to find the host patch at these concentrations. A possible explanation could be that the parasitoid was either under or over stimulated by concentrations below or above 50 aphids/ mL H_2O respectively, became confused and therefore took a longer time to find the host patch under these conditions. It has been noted that under/overstimulation of olfactory organs may lead to increased time for host finding by insect natural enemies and other animals (Palanichamy *et al*., 2019; Kavitha and Dharma, 2014; Fortes-Marco *et al*., 2013).

Table – 1. Mean (± SE) leaf and host arrival times and first escape time of *Lysiphlebus testaceipes* exposed to six concentrations of *Toxoptera citricida* host extracts

Host extract concentration (aphids/mL H_2O)	Mean time ± SE (95% CI)*		
	Leaf arrival time (s)	Host arrival time (s)	First escape time (s)
5	336.00 ± 68.23 (181.64, 490.36) ^{a}	336.00 ± 68.23 (181.64, 490.36) ^{a}	240.00 ± 26.83 (179.30, 300.70) ^{a}
10	342.00 ± 92.11 (133.64, 515.36) ^{a}	348.00 ± 61.84 (208.11, 487.89) ^{a}	576.00 ± 94.00 (340.74, 811.26) ^{bc}
25	360.00 ± 98.44 (114.68, 605.32) ^{ab}	438.00 ± 98.83 (191.81, 484.19) ^{a}	660.00 ± 59.50 (299.19, 620.81) ^{ac}
50	204.00 ± 41.18 (110.84, 297.16) ^{a}	174.00 ± 31.56 (102.61, 245.39) ^{a}	354.00 ± 56.18 (226.92, 481.08) ^{ac}
100	282.00 ± 57.31 (152.36, 411.64) ^{a}	390.00 ± 93.63 (155.56, 424.44) ^{a}	588.00 ± 94.98 (373.11, 802.89) ^{bc}
200	660.00 ± 63.53 (522.29, 809.71) ^{bc}	192.00 ± 33.23 (116.84, 267.16) ^{a}	540.00 ± 79.54 (292.19, 787.81) ^{ac}
Control	723.00 ± 74.82 (627.55, 930.42) ^{bc}	593.00 ± 71.55 (501.67, 892.01) ^{b}	153.00 ± 66.87 (127.33, 177.11) ^{d}

* Values followed by the same letter along a column are not significantly different (P>0.05) from each other based on Tukey-Kramer's Multiple Comparisons test

Host arrival time, the period between *L. testaceipes* introduction and first contact with the host, was not significantly different among the different host extract concentrations tested ($F_{5, 59}$ = 1.98, P>0.05) despite arrival time being the fastest (174.00 ± 31.56 s) at 50 aphids/ mL H_2O. However, *L. testaceipes* arrival times were all significantly shorter (P<0.05) at all concentrations compared to the control (Table 1). The relationship between host arrival time and extract concentrations was best described by the equation $y = 454.68 – 9.02x + 0.13x^2 - 0.0004x^3$ ($R^2 = 0.86$) (Fig. 2).

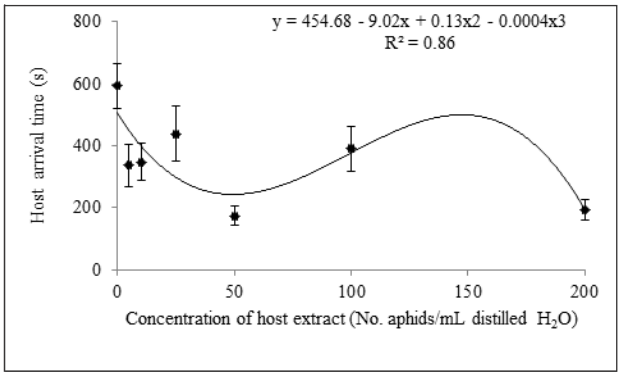

Fig. 2. Host arrival time by *Lysiphlebus testaceipes* exposed to different concentrations of its host extracts

The period between *L. testaceipes* introduction and first escape from the host patch, that is, the first escape time at all host extract concentrations tested was significantly different from the control ($F_{5, 59}$ = 2.54, P<0.05) (Table 1). *L. testaceipes* stayed the longest (660.00 ± 59.50 s) on the host patch at 25 aphids/ mL H_2O though this was not significantly longer (P>0.05) than the time spent by the parasitoid exposed to host concentration of 50 aphids/ mL H_2O (Table 1). The relationship between first escape time and host extract concentration is described by the regression model $y = 400.1 + 2.68x - 0.014x^2 + 0.00002x^3$ which explained 61% of the variation observed (Fig. 3).

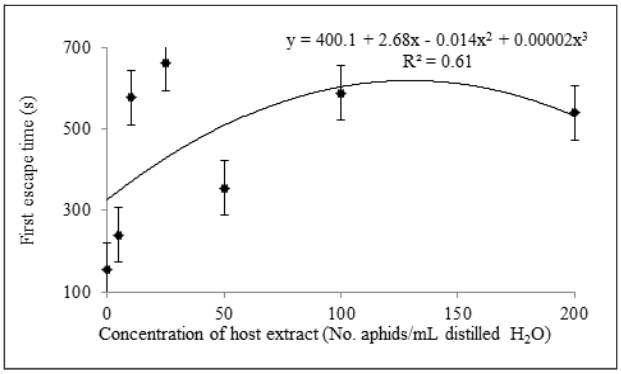

Fig. 3 Mean time of first escape by *Lysiphlebus testaceipes* exposed to different concentrations of its host extracts

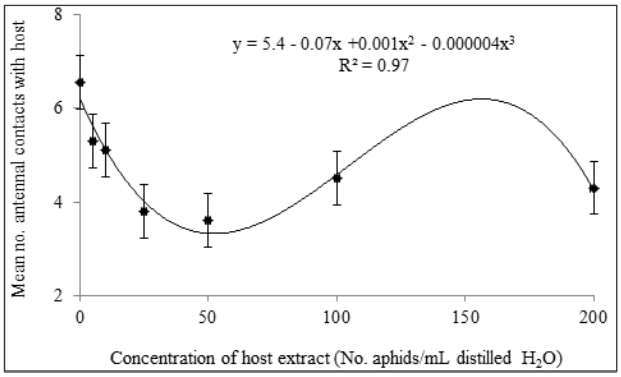

Fig. 4. Mean number of antennal contacts by *Lysiphlebus testaceipes* exposed to different concentrations of its host extracts.

The mean number of antennal contacts with the host and host extract concentration indicated a very high correlation between both parameters (R^2 = 0.97) (Fig. 4). The parasitoid made significantly more antennal contacts (6.55 ± 0.57) with its host at 0 aphids/ mL H_2O (control) compared to the other concentrations ($F_{5,59}$ = 1.26, P>0.05) (Table 2). While this may initially seem contradictory, lower concentrations of host kairomones may necessitate more antennal contact in order to elicit a strong positive response compared to intermediate and higher host concentrations (Table 2).

Table 2 Mean (± SE) number of antennal and oviposition contacts and total time spent in contact with host by *Lysiphlebus testaceipes* exposed to six concentrations of *Toxopteracitricida* extracts.

Host extract concentration (aphids/mL H_2O)	Mean ± SE (95% CI)*		
	No. antennal contacts	No. oviposition contacts	Total time (s) in contact with host
5	5.30 ± 0.54 (4.08, 6.52) ^{ab}	2.20 ± 0.29 (1.54, 2.86)^{ac}	630.00 ± 67.08 (478.25, 781.75) ^{ad}
10	5.10 ± 0.89 (3.09, 7.11) ^{ab}	2.70 ± 0.30(2.02, 3.38)^{a}	1104.00 ± 50.75 (989.19, 1218.81) ^{b}
25	3.80 ± 0.42 (2.86, 4.74) ^{a}	4.60 ± 0.34 (3.83, 5.17) ^{bd}	1074.00 ± 93.57 (862.32, 1285.68) ^{b}
50	3.60 ± 0.75 (1.91, 5.29) ^{a}	3.40 ± 0.52 (2.22, 4.58) ^{ad}	978.00 ± 98.01 (665.82, 1290.18) ^{ab}
100	4.50 ± 0.45 (3.47, 5.53) ^{a}	5.00 ± 0.71 (3.39, 6.62) ^{bd}	966.00 ± 94.14 (708.11, 1223.89) ^{ab}
200	4.30 ± 0.44 (3.34, 5.26)^{a}	5.30 ± 0.79 (3.51, 7.09)^{bd}	1386.00 ± 54.00 (1263.84, 1508.16)^{c}
Control	6.55 ± 0.57 (5.72, 8.89)^{b}	1.90± 0.37 (1.44, 2.01)^{c}	510.00 ± 67.20 (453.19, 653.87)^{d}

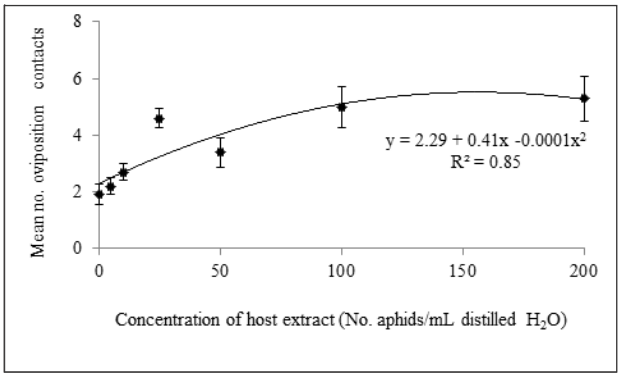

Fig.5. Mean number of oviposition contacts by *Lysiphlebus testaceipes* exposed to different concentrations of its host extracts.

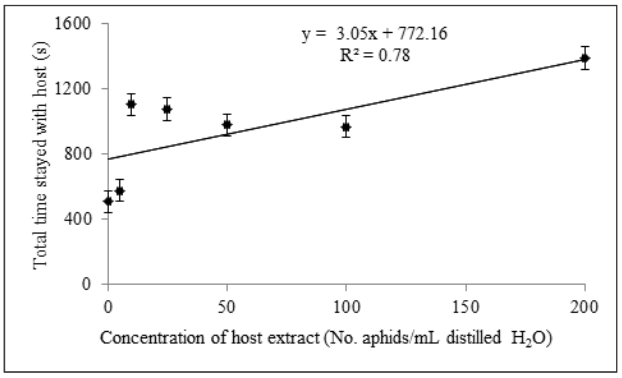

Fig. 6. Mean total time stayed with host by *Lysiphlebus testaceipes* exposed to different concentrations of its host extracts

The mean number of oviposition pricks gradually increased with increasing host extract concentration with a high correlation (R^2= 0.85) between both parameters. Significantly more hosts were ultimately oviposited in at higher host extract concentrations ($F_{5, 59}$ = 5.83, P<0.05) with the number of oviposition pricks eventually beginning to stabilize at a concentration of 100 aphids/ mL H_2O (Table 2 and Fig. 5). This is in compliance with that concluded by Dauphin *et al.* (2009) who suggested that female *Trissolcus basalis* (Wollaston) (Hymenoptera:Scelionidae) visited higher density (concentration) kairomones patches and attacked its host's eggs with greater frequency compared to lower density kairomones patches.

The mean total time spent by *L. testaceipes* in contact with *T. citricida* increased approximately linearly (y =3.05x + 772.16, R^2 = 0.78) with increasing host extract concentrations and was significantly different from the control ($F_{5, 59}$ = 7.10, P<0.05) (Table 2 and Fig. 6). This indicated that *L. testaceipes* females spent more time on the host patch with higher host numbers (=higher host extract concentrations), leading to more of the hosts being found and eventually being parasitized. The increase in oviposition pricks, decrease in antennal contacts, and general decrease in leaf arrival times with increase in host extract concentration, suggests that *L. testaceipes* may be using kairomones from its host as a short-range cue as indicated by Grasswitz and Paine (1992). During the conduct of these experiments, *T. citricida* was often seen using its' hind legs to kick an attacking *L. testaceipes*. When a wasp tapped the host aphid with its antennae, it was sometimes sprayed on the antennae or over the entire head with a liquid from the siphunculi. After an attack some aphids would swing their abdomen to and fro at about 6s intervals while others in close proximity would imitate this behaviour. It is suggested that this maybe how they send out alarm pheromones in response to parasitoid proximity/attack. However, *L. testaceipes* was only deterred for a short time by this behaviour and female parasitoids would return to pricking and ovipositing as soon as the female cleaned off her antennae/head of the siphunculi secretion.

Rutledge (1996) notes that three stages are involved for successful parasitization by parasitoids – habitat location, host location and host acceptance/oviposition. The present study demonstrated that for *L. testaceipes,* all three processes were enhanced by the application of various concentrations its host's (*T. citricida*) extract. The use of kairomones by *L. testaceipes* to enhance host location and levels of parasitization in conservation biological control of *T. citricida* appears to have merit as suggested by Franco *et al.* (2015) for natural enemies of scale insects and Murali-Baskaran *et al.* (2018) in their review.

Conclusions

The study revealed that the behaviour of *L. testaceipes* may be manipulated by the kairomones present in aqueous extracts of its host aphid *T. citricida*. While the application of these kairomones maybe limited under orchard situations, it can be very useful to increase levels of parasitism and hence reduce the spread of Citrus Tristeza Virus (CTV) in a citrus nursery. Additional studies on the active component(s) of the host extract functioning as kairomones may give information about the role of the parasitoid in the regulation of aphid populations in citrus orchards.

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