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Spatial distribution pattern of *Diaphorina citri* Kuwayama (Hem.: Liviidae) on Lime (*Citrus aurantifolia*) and Orange (*Citrus sinensis)* in citrus orchards of Iran

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ABSTRACT

The spatial distribution Pattern of Asian citrus psyllid (ACP), Diaphorina citri, nymphs on Lime and Orange was studied in Jiroft-Bluk section (Kerman Province, Iran) during 2014. The distribution pattern was analyzed using six methods: the indices of variance/mean relationship, coefficient of Green, Lloyd's mean crowding, K index, Iwao's patchiness regression and Taylor's Power Law. The s^2/X *(ranging from 1.98 to 4.89 for lime, and 3.50 to 5.49 for orange); Lloyd's index of patchiness were greater than unity; The Green's index was greater than 0; The K index, in all cases was greater than 0. The slopes of Taylor's power law and Iwao's patchiness regression methods were 1.89 and 1.31 for Lime and 1.45 and 1.30 for Orange, respectively. These slopes had significant difference from one. Based on various mathematical indices of dispersion and regression models, the field dispersion patterns of ACP nymphs were aggregated. Densities of psyllid nymphs were significantly higher on Lime. The population of psyllid nymphs on the tree quadrants did not differ significantly on Lime, however, on orange, psyllid nymphs was also significantly in lower numbers in south quadrant of trees. Densities of psyllid nymphs in the upper half of the canopy were significantly higher than the lower half.*

Key words: Spatial distribution, Population density, Taylor's Power Law, Iwao's patchiness regression

INTRODUCTION

Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hem.: Liviidae) is one of the most serious pest of citrus in the world that transmit the citrus greening agent [1]. This disease was reported in Iran in 2008 [2]. It has been reported from many countries in Asia, Africa and America [3]. It is also reported from southern Iran in 2000 [4].

The spatial distribution pattern is importance in ecological and population sampling theories and also in the development of IPM strategies [5]. For these reasons, a large number of studies have been done in characterizing the spatial distribution of insect populations [6]. Most insect populations have aggregation pattern [7], but the degree of aggregation pattern differs between populations and species [8].

Little is known about the dispersion of the ACP on some citrus plants, such as Orange (*Citrus sinensis* (L.) Osbeck) and there is not study on Lime (*Citrus aurantifolia* (Christm.) Swingle) in Iran and in the world. Soemargono *et al.* (2008) [9] have described the spatial distribution pattern of ACP adults on *Citrus reticulata* var. Madu and orange jasmine, *Murraya paniculata* L., in Malaysia, but their study was done only on adult insects. In the other studies, Xu *et al.*, 1989 (cited in Aubert, 1990) [10] in Fujian (China) and Costa *et al.* (2010) [11] in Brazil (on two 'Valencia' sweet orange) studied the spatial distribution of ACP. In all cases, the spatial distribution of ACP was aggregated. SeTamou *et al.* (2008) [12] found that the number of nymphs in the southeastern quadrant of trees is higher than the other parts of the canopy, in Texas on grapefruit (*Citrus paradisi* Macfad.) and sweet orange (*Citrus sinensis* L); while Soemargono *et al.* (2008) [9] demonstrated that the population of ACP on the different tree quadrants did not differ significantly. Knowledge of spatial distribution parameters of the ACP can be employed to outline a sampling program and to estimate the population density of these insects for use in IPM programs.

In this study several indices of dispersion and regression models is used to determine the spatial distribution pattern of ACP on Lime and Orange trees in Iran. The main questions were: 1) How is the spatial distribution pattern of ACP on lime and orange in south of Iran? 2) Does spatial distribution pattern of *D.citri* related to host plants and to upper and lower halves of tree? 3) Does population density vary between cardinal directions of the canopy?

MATERIALS AND METHODS

Sampling unit and sampling pattern

The number of nymphs per shoot (up to 10 cm in length) in two citrus varieties was selected as a sample unit. According to Soemargono *et al.* (2008) [9], 15 trees of similar size, each of lime and orange in south of Iran (Blook sector, Jiroft, Kerman), were randomly selected and the canopy of each tree was partitioned into two section (upper and lower halves); after that each section divided into four subsection or directions (north, west, south and east). Therefore, there were eight sectors in each tree; subsequence the shoots were randomly chosen in each sector (totally 80 shoots per tree). The number of nymphs per shoots was recorded for each tree in situ [9].

Since sampling unit (SU) were the number of individuals per shoot (up to 10 cm in length) and the total number of shoots was 80 per tree, the data was arranged on the original counts in the form of frequency distribution that is conformed by Ludwig and Reynolds (1988) [13] when the number of SUs is more than 30, the data should be arranged as a frequency distribution [9].

The relative variation of sampling method (*RV*) was calculated according to Hillhouse & Pitre (1974) [14].

Sampling frequency was conducted one other day (48 h interval) from 20th October to 20th December 2014.

Spatial distribution pattern

The spatial distribution of *D. citri* was determined using index of dispersion [15, 16, 17], Taylor's power law [18], Iwao's patchiness regression [19], Lloyd's mean crowding [20], Green's Index(*Cx*) [21] and K Index [22].

Statistical Analysis

An ANOVA procedure was done in SAS software to determine differences among populations among sectors in tree canopies. In this analysis the average number of insects per shoot was used. The split-plot experimental design with the tree as a block or replication (n = 15), the stratum as the main plot (n = 2), and the quadrant as the sub-plot (n = 4) was used.

RESULTS AND DISCUSSION

Data set from primary sampling were employed to calculate *RV*. Calculated *RV* for *D. citri* was 5.6% and 8.85% on orange and lime, respectively.

According to results of the variance to mean ratio (S^2/m), index of dispersion (I_D) and *Z* test , Lloyd's mean crowding , Green's Index and K index, the spatial distribution of nymphal stage of *D. citri* on lime and orange is an aggregated type (Tables 1 and 2). In all cases the s^2/X were > 1 (ranging from 1.98 to 4.89 for lime, and 3.50 to 5.49 for orange); Lloyd's index of patchiness was greater than unity; The calculated *z* in the index of dispersion (I_D) was greater than 1.96, that showed the spatial distribution of *D. citri* in lime and orange as aggregated (Tables 2, 3). The Green's index (C_x) is also presented for both host plants in Tables 2 and 3. In all cases *Cx* is greater than 0, which indicated aggregated distribution. The K index, in all case is greater than 0, (1/k>0) that reveals an aggregated distribution.

The slope values of Taylor's power law (Table 3) on lime and orange were significantly greater than 1 (t = 25.39 and 13.01; df = 14; P < 0.05), indicating clumped or aggregated distributions on these host plants. Similarly, the slopes of Iwao's model (Table 3) on lime and orange were also significantly greater than 1 (t = 26.35 and 3.97; df = 14; P < 0.05), suggesting that the distribution pattern of the citrus psyllids to be aggregated on both host plants. The calculated *t* (t_c) was greater than t-table (t_t), that indicating an aggregated spatial distribution of nymphal stages of *D. citri*. Similar results were found for *D. citri* nymph on Grapefruit and Sweet orange [12], and also on Sweet orange [11].

The Taylor's power law aggregation index for *D. citri* nymphs on Lime (1.89) (Table 1) was higher than the value of 1.72 and 1.76 (on Grapefruit and Sweet orange, respectively) [12] and 1.43 on Sweet orange [11], and also 1.30 and 1.56 on *Citrus reticulata* and orange jasmine, respectively as reported by Soemargono *et al.* (2008) [9], suggesting that *D.citri* is more clumped on Lime than other host plants mentioned above.

Table 1- Statistical distribution and indices of dispersion for *Diaphorina citri* on Lime tree

Tree no.	$S^2/\bar{X}$ ratio	Lloyd's mean crowding	Lloyd's mean to mean ratio	Index of Dispersion (I_D)	Z value For I_D	Green's Index (C_x)	K Index
1	4.46	25.48	1.15	352.50	14.02	0.04	0.63
2	3.85	21.60	1.15	304.48	12.14	0.03	0.56
3	4.87	22.83	1.20	384.80	15.21	0.04	0.66
4	4.89	22.78	1.20	387.02	15.29	0.04	0.67
5	2.38	10.74	1.14	188.46	6.88	0.01	0.35
6	2.66	14.29	1.13	210.91	8.00	0.02	0.40
7	2.00	8.419	1.13	158.56	5.27	0.01	0.27
8	2.18	14.18	1.09	172.61	6.05	0.01	0.32
9	2.47	14.61	1.11	195.73	7.25	0.01	0.37
10	2.32	10.80	1.13	183.39	6.62	0.01	0.34
11	3.43	14.76	1.19	271.44	10.77	0.03	0.51
12	1.98	8.836	1.12	156.96	5.18	0.01	0.27
13	2.29	14.18	1.10	181.25	6.50	0.01	0.34
14	2.81	12.62	1.16	222.53	8.56	0.02	0.42
15	2.75	11.41	1.18	217.53	8.32	0.02	0.41

Table 2- Statistical distribution and indices of dispersion for *Diaphorina citri* on Orange tree

Tree no.	$S^2/\bar{X}$ ratio	Lloyd's mean crowding	Lloyd's mean to mean ratio	Index of Dispersion (ID)	Z value For ID	Green's Index (C_x)	K index
1	4.96	10.26	1.62	391.87	15.46	0.05	0.64
2	4.12	8.78	1.55	325.98	13.00	0.03	0.56
3	4.84	10.45	1.58	382.45	15.12	0.04	0.63
4	4.21	8.26	1.63	332.63	13.26	0.04	0.56
5	4.72	9.59	1.63	373.23	14.79	0.04	0.62
6	5.49	9.51	1.89	434.31	16.94	0.05	0.67
7	3.70	7.97	1.51	292.31	11.64	0.03	0.51
8	4.22	7.60	1.73	333.44	13.29	0.04	0.55
9	4.92	8.68	1.82	388.97	15.36	0.04	0.62
10	3.50	7.20	1.53	277.19	11.01	0.03	0.48
11	3.71	6.80	1.66	293.67	11.70	0.03	0.50
12	4.04	5.89	2.06	319.36	12.74	0.03	0.51
13	3.50	7.27	1.52	277.26	11.01	0.03	0.48
14	3.72	7.29	1.59	294.10	11.72	0.03	0.50
15	3.99	5.96	2.00	315.27	12.58	0.03	0.51

Table 3- Spatial distribution of *Diaphorina citri* on Lime and Orange using Taylor's power law and Iwao's patchiness regression analysis

Hosts	Taylor					Iwao					t_t	
	a	b	SE_b	R_2	t_c	α	β	$SE_β$	R_2	t_c	t_t (0.05)	t_t (0.01)
Lime	-0.57	1.89	0.03	0.93	25.39	-0.41	1.31	0.01	0.98	26.35	2.14	2.94
Orange	0.21	1.45	0.03	0.84	13.01	-0.64	1.30	0.07	0.68	3.97		

Table 4- Statistical distribution and indices of dispersion of *Diaphorina citri* on Upper halve of Lime canopy

Tree no.	$S^2/\bar{X}$ ratio	Lloyd's mean crowding	Lloyd's mean crowding to mean ratio	Index of Dispersion (ID)	Z value For ID	Green's Index (C_x)	K index
1	3.28	32.20	1.07	259.44	10.24	0.029	0.50
2	2.86	27.06	1.07	226.54	8.75	0.023	0.44
3	3.59	28.27	1.10	284.20	11.31	0.032	0.54
4	3.51	28.86	1.09	277.41	11.02	0.031	0.53
5	1.98	12.21	1.08	157.19	5.20	0.012	0.28
6	2.01	17.71	1.06	159.44	5.32	0.012	0.29
7	1.60	9.85	1.06	127.06	3.41	0.007	0.19
8	1.73	17.10	1.04	137.03	4.02	0.009	0.22
9	2.01	17.16	1.06	159.22	5.31	0.012	0.29
10	2.24	11.06	1.12	177.09	6.29	0.015	0.32
11	2.59	17.89	1.09	205.24	7.73	0.020	0.39
12	2.16	8.366	1.16	171.13	5.97	0.014	0.30
13	1.76	17.51	1.04	139.46	4.17	0.009	0.23
14	2.37	14.22	1.10	187.25	6.82	0.017	0.35
15	2.10	13.75	1.08	166.15	5.69	0.013	0.30

Table 5- Statistical distribution and indices of dispersion of *Diaphorina citri* on Lower halve of Lime canopy

Tree no.	$S^2/\bar{X}$ ratio	Lloyd's mean crowding	Lloyd's mean crowding to mean ratio	Index of Dispersion (ID)	Z value For ID	Green's Index (C_x)	K index
1	6.95	20.08	1.42	549.66	20.62	0.075	0.81
2	5.87	17.17	1.39	464.14	17.93	0.061	0.74
3	7.54	18.79	1.53	595.66	21.98	0.082	0.84
4	8.09	18.52	1.62	639.82	23.24	0.089	0.87
5	2.97	9.478	1.26	235.26	9.16	0.025	0.43
6	3.94	11.49	1.34	311.43	12.42	0.037	0.56
7	2.66	7.243	1.29	210.83	8.00	0.021	0.38
8	2.95	11.57	1.20	233.14	9.06	0.024	0.44
9	3.21	12.33	1.21	253.97	10.00	0.028	0.47
10	2.40	10.55	1.15	190.16	6.97	0.017	0.35
11	5.07	12.42	1.48	400.66	15.77	0.051	0.66
12	1.83	9.334	1.09	144.96	4.49	0.010	0.24
13	3.27	11.30	1.25	258.83	10.22	0.028	0.48
14	3.47	11.24	1.28	274.20	10.88	0.031	0.50
15	3.98	9.660	1.44	314.88	12.56	0.037	0.55

Table 6- Statistical distribution and indices of dispersion of *Diaphorina. citri* on Upper halve of Orange canopy

Tree no.	$S^2/\bar{X}$ ratio	Lloyd's mean crowding	Lloyd's mean crowding to mean ratio	Index of Dispersion (ID)	Z value For ID	Green's Index (C_x)	K index
1	4.02	11.07	1.37	317.61	12.67	0.038	0.56
2	3.38	9.93	1.31	267.44	10.59	0.030	0.49
3	3.69	11.01	1.32	291.81	11.62	0.034	0.53
4	3.31	8.73	1.35	261.50	10.33	0.029	0.47
5	3.69	10.19	1.35	291.99	11.63	0.034	0.52
6	4.32	9.35	1.55	341.90	13.61	0.042	0.58
7	3.20	8.68	1.34	253.54	9.988	0.027	0.46
8	3.24	7.76	1.40	256.14	10.10	0.028	0.46
9	4.05	9.10	1.50	320.58	12.79	0.038	0.55
10	2.96	7.93	1.32	233.94	9.100	0.024	0.42
11	3.22	7.60	1.41	254.88	10.04	0.028	0.45
12	3.39	6.01	1.66	268.01	10.62	0.030	0.45
13	3.41	8.34	1.40	269.93	10.70	0.030	0.48
14	3.55	8.05	1.46	280.86	11.17	0.032	0.49
15	2.97	5.64	1.53	234.97	9.148	0.024	0.40

By homogeneity tests on the regression slopes, it was found that there was no significant difference between the slopes of Taylor's power law ($t = 0.142$; $df = 26$; $P > 0.05$) for two host plants. There was also no significant difference for the psyllid nymph population on two host plants when the Iwao's regression model was used ($t = 0.170$; $df = 26$; $P > 0.05$) (Figures 1 and 2). Therefore, all data were pooled and an overall regression was plotted. In general, Taylor's power law fitted the data better than the Iwao's model on Orange, but on lime the Iwao's model fitted the data better than the Taylor's power law. This is based on the higher values of R^2 for each model.

We also determined all above mentioned indices separately for the two strata; upper and lower halves of Lime and Orange canopy. Results showed that according to variance / mean ratio (S^2/m), index of dispersion (I_D) and *Z* test, Lloyd's mean crowding, Green's Index and K index, the spatial distribution of nymphal stages of *D.citri* on upper and lower halves of Lime and Orange canopy was an aggregated type (Tables 4, 5, 6 and 7). But, the slopes of Iwao's model (Table 8) on lower halve of orange was less than 1, suggesting that the distribution pattern of the citrus psyllids to be uniform, but the positive value of α indicates the tendency to crowding of population in space. In other hand, the slope values of Taylor's power law (Table 8) for the lower halve of orange, was greater than 1, indicating clumped or aggregated distributions on this halve. Of course, the Taylor's power law fitted the data better than the Iwao's model (R^2 value in Table 8) and also according to other indexes (Tables 4, 5, 6 and 7), it is concluded that the spatial distribution of *D.citri* on upper and lower halves of lime and orange canopy are similar and is an aggregated type.

Based on the analysis of variance, distribution of *D.citri* population on lime trees, was not significantly different among cardinal directions (north, west, south and east) of tree canopy, suggesting that there is a high density of this pest in all tree's quadrants. These results were similar to those obtained by Soemargono *et al.* (2008) [9] on *Citrus reticulata* and orange jasmine. But there is significant difference (Table 9) between psyllid densities in upper and lower halves of trees, where the upper canopy harbored more psyllids than the lower half. Higher density of *D.citri* on upper halve of citrus can be related to more younger shoots in upper halve of the tree and their preference for new flushes for feeding and development. On the other hand, significant difference of the pest population density was observed among cardinal directions of orange tree canopy. Distribution of *D.citri* population in south quadrant is less than other quadrants in upper and lower halves of orange tree (Table 9). In general, the psyllid distribution was significantly higher at the upper halve of the canopies (Table 10). It was found that the density of *D.citri* in lime is higher than orange tree (Table 10). The higher density of *D.citri* on lime might be related to the increase in ratios of nutritious necessary for the growth of developmental stages.

Table 7- Statistical distribution and indices of dispersion of *Diaphorina citri* on Lower halve of Orange canopy

Tree no.	$S^2/\bar{X}$ ratio	Lloyd's mean crowding	Lloyd's mean crowding to mean ratio	Index of Dispersion (ID)	Z value For ID	Green's Index (C_x)	K index
1	6.95	20.08	1.42	549.66	20.62	0.075	0.81
2	5.87	17.17	1.39	464.14	17.93	0.061	0.74
3	7.54	18.79	1.53	595.66	21.98	0.082	0.84
4	8.09	18.52	1.62	639.82	23.24	0.089	0.87
5	2.97	9.47	1.26	235.26	9.161	0.025	0.43
6	3.94	11.49	1.34	311.43	12.42	0.037	0.56
7	2.66	7.24	1.29	210.83	8.004	0.021	0.38
8	2.95	11.57	1.20	233.14	9.063	0.024	0.44
9	3.21	12.33	1.21	253.97	10.00	0.028	0.47
10	2.40	10.55	1.15	190.16	6.97	0.017	0.35
11	5.07	12.42	1.48	400.66	15.77	0.051	0.66
12	1.83	9.33	1.09	144.96	4.49	0.010	0.24
13	3.27	11.30	1.25	258.83	10.22	0.028	0.48
14	3.47	11.24	1.28	274.20	10.88	0.031	0.50
15	3.98	9.66	1.44	314.88	12.56	0.037	0.55

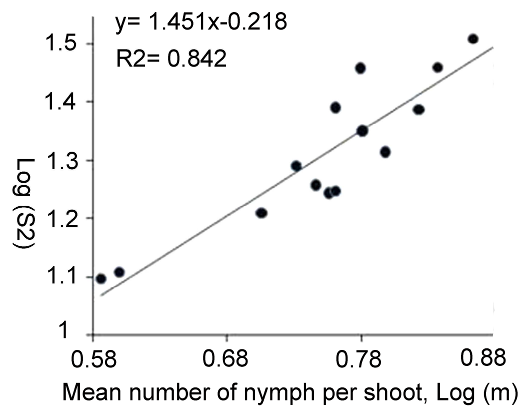
Table 8- Spatial distribution of *Diaphorina citri* on upper and lower halves of Lime and Orange canopy using Taylor's power law and Iwao's patchiness regression analysis

Host plant	halves	Taylor					Iwao					t_t	
		a	b	SE_b	R^2	t_c	α	β	$SE_β$	R^2	t_c	t_t (0.05)	t_t (0.01)
Lime	Upper	-0.24	1.48	0.05	0.91	9.49	-0.38	1.30	0.01	0.98	29.2	2.13	2.94
	Lower	-0.81	2.35	0.05	0.71	25.8	-0.32	1.21	0.03	0.91	5.84		
Orange	Upper	0.18	1.34	0.04	0.81	8.72	-0.57	1.33	0.05	0.85	6.46	2.13	2.94
	Lower	0.31	1.54	0.03	0.82	16.1	0.01	0.78	0.11	0.25	-1.86		

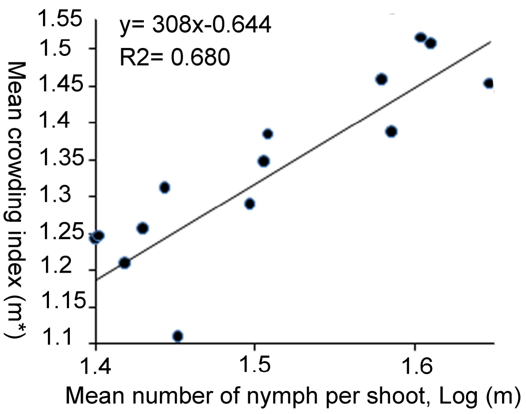
Table 9- Population density of *Diaphorina. citri* (mean No. of nymphs of *psyllid* per shoot) on Lime and orange with respect to quadrant and stratum

Quadrant	lime		Orange	
	Upper	Lower	Upper	Lower
North	17.24 ^{a}	9.22 ^{b}	6.30 ^{a}	5.66 ^{a}
West	18.68 ^{a}	10.56 ^{b}	7.40 ^{a}	4.06 ^{b}
South	12.94 ^{a}	8.49 ^{b}	3.77 ^{b}	1.21 ^{c}
East	18.44 ^{a}	9.57 ^{b}	7.05 ^{a}	3.60 ^{b}

Means within the respective columns followed by the same letter are not significantly different at P = 0.01 according to LSD.

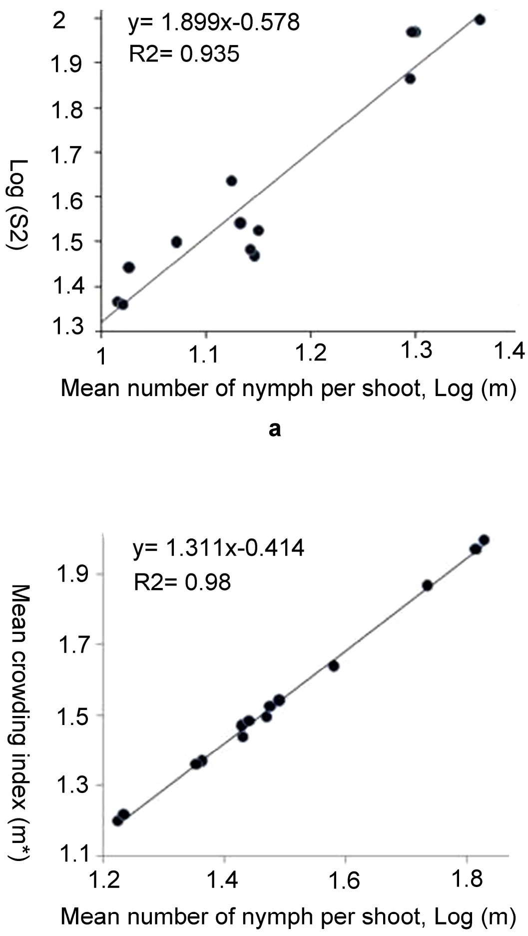


a



b

Figure 1- Regression analysis of (a) Taylor's power law, and (b) Iwao's model for *Diaphorina citri* on total pooled samples (Orange)



b

Figure 2- Regression analysis of (a) Taylor's power law and (b) Iwao's model for *Diaphorina citri* on total pooled samples (Lime)

Table 10- Population density of. *Diaphorina citri* (mean No. of psyllid nymphs per shoot) on Lime and orange with respect to each host plant

Host		density on halves	density on each Host
Lime:	Upper	16.82 [a]	13.20 [a]
	Lower	9.57 [b]	
Orange:	Upper	6.13 [a]	4.28 [b]
	Lower	3.53 [b]	

Means within the respective rows followed by the same letter are not significantly different at P = 0.05 according to t-test.

The causes of aggregation in these psyllids might be due to their inherent active aggregative behavioral response such as in a situation where the presence of one individual attracts the others, perhaps for the purpose of feeding and reproduction, and also due to some heterogeneity of the environment such as microclimate and preferred part of the plant [7, 23, 24]. It was found that the ovipositing and feeding sites of *D.citri* are always on young flushes. The difference in mean counts of *D.citri* between upper and lower halves of the host plant canopy and also between quadrants seemed to be highly influenced by physical factors such as exposure to the sun lights.

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