

## Comparison of Different Sampling Procedures for Population Monitoring of Important Citrus Aphids on Two Orange Species

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### ABSTRACT

Sampling is a key aspect of integrated pest management program. In this study, different sampling procedures for population monitoring of three important citrus aphids, *Aphis spiraecola*, *A. gossypii* and *Toxoptera aurantii* were compared on two citrus trees, Satsuma mandarin and Thomson navel orange, in order to determine the most appropriate one. The samplings were performed from different heights, main directions and inner or outer foliage layer of the trees. Also, efficacy of two traps, yellow sticky and yellow basin traps, for monitoring of the aphids were evaluated. Results showed that the best sampling procedures were different according to aphid and host plant species. Except *T. aurantii* on Satsuma mandarin, both traps were not efficient for estimating population of the aphids under field condition. The findings can be used in an IPM program of the aphids in citrus orchards.

*Key words:* Aphididae, host plants, traps, IPM.

## INTRODUCTION

Citrus (Family: Rutaceae) is one of the world's major fruit crops with global availability and popularity in human diets (Liu, Heying, Tanumihardjo, 2012). Citrus pests are important problem confronting the citrus grower in Iran (Farahbakhsh, 1996). Aphids (Hom., Aphididae) are important citrus pests in Iran (Rajabi, 1986). Large amount of broad spectrum insecticides (BSIs) are applied to control the pests in north of Iran (Farahbakhsh, 1996). BSIs cause many problems including reduced profits from high insecticide costs, destruction of non-target organism, development of resistance in populations, pest replacement, pest resurgence, environment pollution and etc. (Pedigo, 2002). Establishment of suitable integrated pest management (IPM) program is critical for sustainable pest control and reduction of BSIs in citrus orchards (Farahbakhsh, 1996). Sampling for decision making is a key aspect of IPM. Due to cost and time consuming of sampling, growers must know how to gather enough information about pest abundance to able make precise decisions without incurring excessive costs. Selection of the best sampling procedure has a crucial role in sampling program (Binns & Nyrop, 1992; Pedigo, 2002). Estimation of population densities in highly aggregated insect species in complex and variable habitats can be difficult to estimate efficiently, accurately, and with minimal variance (Whitaker, Mahr, & Clayton, 2006). Appropriate sampling procedure is especially important in situations where heterogeneity in pest density is suspected (Binns & Nyrop, 1992). Also, traps as sampling tools were extensively used to estimate population density (Pedigo, 2002). Among traps, yellow sticky trap (Heathcote, 1957) and yellow water trap (Heathcote, 1957; O'Loughlin, 1963) were previously applied to population monitoring of some aphid species. There have not been made any effort to determine the best sampling procedure for population monitoring of citrus aphids. Therefore, the objectives of the present experiment were selecting the best sampling procedures of various citrus aphids and efficacies of two traps, yellow sticky and water traps, for population monitoring of the aphids.

## MATERIAL AND METHODS

The experiments were performed in an experimental citrus orchard of citrus and subtropical fruits research center, 20 hectares, in Ramsar, Mazandaran province, north of Iran, 36°54'24.2"N 50°39'26.7"E from January 2016 to August 2017. No pesticides were applied during the study period.

### Sampling

Samplings for estimating aphid density were weekly performed. At each sampling date, ten Thompson navel orange, *Citrus sinsensis* L. (20 years old), and ten Satsuma mandarin, *Citrus unshiu* Markovich, trees (20 years old) were randomly chosen. Different samples were collected at three heights (1, 1.5 and 2 meters), four directions (north, south, east and west) and on two foliage layers (inner and outer layers). From each sampling procedure, one shoot and totally 24 shoots from each tree, were randomly taken. The samples were separately transferred to the

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entomological laboratory and number of each species was separately recorded under stereomicroscope. For monitoring of alate aphid population, two types of traps were used. 1- Yellow sticky trap (30×10 cm) (Russel IPM, UK) and 2- yellow basin trap (25 cm in diameter) which was half filled with water + detergent. Eight of each traps was used per hectare and number of trapped aphids was weekly recorded.

### Data analysis

Regression analysis between aphid numbers of each sampling procedure and total aphid number on each tree were used to select the best sampling procedure. The regression between mean aphids of each trap and mean aphids per tree were performed to investigate efficacy of the traps for population monitoring,. All analyses were done by SPSS (version 16.0) software.

## RESULTS

Results of regression analysis for selecting the best foliage layer (inner or outer) of Satsuma mandarin and Thomson navel orange for population monitoring of *Aphis spiraecola* Patch, *A. gossypii* Glover and *Toxoptera aurantii* Boyer de Fonscolombe are showed in table 1. There was a significant correlation between the population of both layers and total aphid population on Satsuma mandarin. Therefore, both foliage layers can be used for monitoring the aphids of Satsuma mandarin. But, there were not significant regression between density of *A. gossypii* collected from inner layer and total density of Thomson navel orange. Hence, inner foliage layer of the orange is not suitable sampling procedure. Also, results of selection of the best height for sampling *A. spiraecola*, *A. gossypii* and *T. aurantii* on Satsuma mandarin and Thomson navel orange are presented in table 2. The results of the present experiment imply that an estimate of population density of the aphids in Satsuma mandarin can be obtained regardless of the height at which the traps are positioned. Similarly, significant correlations were found between aphid density of each heights and total population on Thomson navel orange. The only exception was *T. aurantii* on 2m height of Thomson navel foliage which did not provide a good estimate of the population density.

There were significant correlations between the aphids population from each main direction (north, south, west and east) and total population (Table 3). The data showed that each main direction could be used for monitoring of *A. spiraecolae*, *A. gossypii* and *T. aurantii*. But there was not significant correlation between *T. aurantii* in south direction of Thomson navel orange and total density of the aphid. Therefore, the sampling procedure is not appropriate for the aphid sampling.

Regression analysis for evaluating efficacy of the yellow sticky trap and the yellow basin trap are showed in table 4 and 5, respectively.

The results showed that both traps, yellow sticky and yellow basin traps, could be used to monitor *T. aurantii* in Satsuma mandarin. There were no significant correlations between the trapped individuals of other aphid species on Satsuma mandarin. Also, both traps are not suitable for monitoring of all species in Thomson navel orange.

Table 1. Regression analysis for selecting the best sampling procedure in inner and outer layer of the Satsuma mandarin and Thomson navel orange foliage.

	Aphid species	Foliage layer	N	Intercept±SE	Regression slope LINE±SE	R <sup>2</sup>	F <sub>(df)</sub>	P <sub>regression</sub>
Satsuma mandarin	<i>A. spiraeocolae</i>	Outer	35	0.13±0.158	1.415±0.036	0.979	1521 <sub>(1,33)</sub>	<0.0001
		Inner	35	-0.13±0.158	0.585±0.036	0.888	260.4 <sub>(1,33)</sub>	<0.0001
	<i>A. gossypii</i>	Outer	35	-0.022±0.025	0.0395±0.016	0.948	619.4 <sub>(1,33)</sub>	<0.0001
		Inner	35	0.022±0.025	1.605±0.016	0.997	10235.2 <sub>(1,33)</sub>	<0.0001
	<i>T. aurantii</i>	Outer	35	-0.007±0.015	1.903±0.042	0.984	2082.5 <sub>(1,33)</sub>	<0.0001
		Inner	35	0.007±0.015	0.097±0.042	0.142	5.4 <sub>(1,33)</sub>	<0.026
Thomson navel orange	<i>A. spiraeocolae</i>	Outer	35	0.03±0.026	1.734±0.026	0.992	4327.8 <sub>(1,33)</sub>	<0.0001
		Inner	35	-0.017±0.022	0.278±0.024	0.809	139.9 <sub>(1,33)</sub>	<0.0001
	<i>A. gossypii</i>	Outer	35	0.0±0.001	1.996±0.005	1	185325	<0.0001
		Inner	35	0.001±0.001	0.004±0.005	0.158	0.85	0.363
	<i>T. aurantii</i>	Outer	35	3.2×10 <sup>-11</sup> ±0	2±0	1	-	-
		Inner	35	-	-	-	-	-

Table 2. Regression analysis for selection of the best sampling procedure in different heights of Satsuma mandarin and Thomson navel orange foliage.

	Aphid species	Foliage height	N	Intercept±SE	Regression slope LINE±SE	R <sup>2</sup>	F <sub>(df)</sub>	P <sub>regression</sub>	
Satsuma mandarin	<i>A. spiraeocolae</i>	1 m	34	0.043±0.053	0.4±0.012	0.971	1076.3 <sub>(1,32)</sub>	>0.0001	
		1.5 m	34	-0.0108±0.14	1.08±0.032	0.973	1132.2 <sub>(1,32)</sub>	>0.0001	
		2 m	34	0.065±0.147	1.511±0.034	0.984	1991.3 <sub>(1,32)</sub>	>0.0001	
	<i>A. gossypii</i>	1 m	34	0.018±0.012	0.295±0.008	0.978	1437.1 <sub>(1,32)</sub>	>0.0001	
		1.5 m	34	-0.029±0.021	1.052±0.013	0.995	6611 <sub>(1,32)</sub>	>0.0001	
		2 m	34	0.011±0.02	1.653±0.012	0.998	17702 <sub>(1,32)</sub>	>0.0001	
	<i>T. aurantii</i>	1 m	35	0.001±0.047	1.478±0.129	0.8	131.6 <sub>(1,33)</sub>	>0.0001	
		1.5 m	35	-0.001±0.013	0.514±0.037	0.855	194.8 <sub>(1,33)</sub>	>0.0001	
		2 m	35	0.0±0.034	1.008±0.092	0.784	120 <sub>(1,33)</sub>	>0.0001	
Thomson navel orange	<i>A. spiraeocolae</i>	1 m	35	-0.086±0.07	0.995±0.074	0.847	183.1 <sub>(1,33)</sub>	>0.0001	
		1.5 m	35	0.067±0.062	0.29±0.065	0.375	19.9 <sub>(1,33)</sub>	>0.0001	
		2 m	35	0.018±0.066	1.715±0.069	0.949	619.6 <sub>(1,33)</sub>	>0.0001	
	<i>A. gossypii</i>	1 m	35	0.005±0.006	0.443±0.034	0.837	169.6 <sub>(1,33)</sub>	>0.0001	
		1.5 m	35	-0.003±0.016	1.131±0.098	0.802	133.57 <sub>(1,33)</sub>	>0.0001	
		2 m	35	-0.002±0.016	1.42±0.96	0.869	218.4 <sub>(1,33)</sub>	>0.0001	
	<i>T. aurantii</i>	1 m	35	-	-	-	-	>0.0001	
		1.5 m			-8.9×10 <sup>-5</sup> ±0	3±0.021	0.998	2079.1 <sub>(1,33)</sub>	>0.0001
		2 m	35		8.9×10 <sup>-5</sup> ±0	0±0.021	0	0 <sub>(1,33)</sub>	0.989

Table 3. Regression analysis for selection of the best sampling procedure in different direction (North, south, west and east) of Satsuma mandarin and Thomson navel orange foliage.

	Aphid species	Direction	N	Intercept±SE	Regression slope LINE±SE	R <sup>2</sup>	F <sub>(df)</sub>	P <sub>regression</sub>
Satsuma mandarin	<i>A. spiraeocolae</i>	North	35	-0.027±0.069	0.568±0.016	0.976	1312.2 <sub>(1,33)</sub>	>0.0001
		South	35	0.118±0.152	0.789±0.034	0.943	527.8 <sub>(1,33)</sub>	>0.0001
		East	35	0.165±0.265	1.311±0.06	0.937	478.6 <sub>(1,33)</sub>	>0.0001
		West	35	0.074±0.155	1.331±0.35	0.978	1452 <sub>(1,33)</sub>	>0.0001

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Table 3. Continued.

	Aphid species	Direction	N	Intercept±SE	Regression slope LINE±SE	R <sup>2</sup>	F <sub>(df)</sub>	P <sub>regression</sub>
Satsuma mandarin	<i>A. gossypii</i>	North	35	-0.055±0.5	0.909±0.039	0.944	557.1 <sub>(1,33)</sub>	>0.0001
		South	35	0±0	1±0	1	-	-
		East	35	0.029±0.075	1.128±0.058	0.919	376.8 <sub>(1,33)</sub>	>0.0001
		West	35	-0.082±0.071	1.746±0.055	0.968	1009.1 <sub>(1,33)</sub>	>0.0001
	<i>T. aurantii</i>	North	35	0.001±0.107	1.471±0.301	0.419	23.8 <sub>(1,33)</sub>	>0.0001
		South	35	0.001±0.066	1.25±0.186	0.579	45.33 <sub>(1,33)</sub>	>0.0001
		East	35	0.001±0.009	0.388±0.025	0.88	241 <sub>(1,33)</sub>	>0.0001
		West	35	-0.003±0.035	0.388±0.025	0.712	81.6 <sub>(1,33)</sub>	>0.0001
Thompson navel orange	<i>B. spiraeocolae</i>	North	35	-0.001±0.49	0.725±0.051	0.858	199.2 <sub>(1,33)</sub>	>0.0001
		South	35	0.062±0.075	1.072±0.079	0.848	184.57 <sub>(1,33)</sub>	>0.0001
		East	35	-0.012±0.077	1.216±0.081	0.83	227.1 <sub>(1,33)</sub>	>0.0001
		West	35	0.076±0.86	0.987±0.091	0.781	117.7 <sub>(1,33)</sub>	>0.0001
	<i>A. gossypii</i>	North	35	0.0±0.017	1.267±0.108	0.806	137.1 <sub>(1,33)</sub>	>0.0001
		South	35	0.001±0.013	0.83±0.8	0.766	108.3 <sub>(1,33)</sub>	>0.0001
		East	35	-0.001±0.007	0.99±0.046	0.934	467.8 <sub>(1,33)</sub>	>0.0001
		West	35	0.001±0.014	0.913±0.085	0.778	115.5 <sub>(1,33)</sub>	>0.0001
	<i>T. aurantii</i>	North	35	7.27×10 <sup>-2</sup> ±0.002	1.77±0.357	0.428	24.65 <sub>(1,33)</sub>	>0.0001
		South	35	0.0±0.0	0.0±0.28	0	0 <sub>(1,33)</sub>	0.989
		East	35	-	-	-	-	-
		West	35	0.0±0.002	2.22±0.357	0.542	38.9 <sub>(1,33)</sub>	>0.0001

Table 4. Regression between mean densities of citrus aphids on Satsuma mandarin and Thomson navel orange trees and trapped aphids by yellow sticky traps.

	Aphid species	N	Intercept±SE	Regression slope LINE±SE	R <sup>2</sup>	F <sub>(df=1,5)</sub>	P <sub>regression</sub>
Satsuma mandarin	<i>A. spiraeocolae</i>	7	2.502±2.26	0.494±0.26	0.419	3.599	0.116
	<i>A. gossypii</i>	7	2.52±1.32	0.486±0.376	0.251	1.675	0.252
	<i>T. aurantii</i>	7	0.332±0.464	2.039±0.601	0.697	11.513	0.019
Thompson navel orange	<i>A. spiraeocolae</i>	7	10.96±9.79	10.01±36.3	0.015	0.076	0.794
	<i>A. gossypii</i>	7	10.5±6.42	1.36±1.82	0.101	0.56	0.488
	<i>T. aurantii</i>	7	-	-	-	-	-

Table 5. Regression between mean densities of citrus aphids on Satsuma mandarin and Thomson navel orange trees and trapped aphids by yellow basin traps.

	Aphid species	N	Intercept±SE	Regression slope LINE±SE	R <sup>2</sup>	F <sub>(df=1,5)</sub>	P <sub>regression</sub>
Satsuma mandarin	<i>A. spiraeocolae</i>	7	11.15±7.03	1.266±0.839	0.313	2.278	0.192
	<i>A. gossypii</i>	7	10.5±6.42	1.362±1.828	0.101	0.56	0.488
	<i>T. aurantii</i>	7	0.055±0.055	1.277±0.071	0.985	320.8	<0.0001
Thompson navel orange	<i>A. spiraeocolae</i>	7	10/96±9.79	10.01±36.3	0.015	0.076	0.794
	<i>A. gossypii</i>	7	4.46±5.4	21.8±15.45	0.286	2.002	0.216
	<i>T. aurantii</i>	7	-	-	-	-	-

## DISCUSSION

Our finding indicated the best sampling procedure (from different heights, main directions and inner or outer layer foliage) for population estimating of three important citrus aphids on two citrus trees, Satsuma mandarin and Thomson navel orange. The best sampling procedures were different according to aphid or host plant species. The differences may be due to different behavior of various aphid species on same or different host plants. Janzen (1973) demonstrated that many factors including host plant and pest species, seasons, time of day and etc influences sampling program. Our finding agrees with Hajek & Dahlsten (1986) who showed that three coexisted aphids select different ecological niche for feeding on *Betula pendula* Roth. Similarly, it is demonstrated *Aphis pomi* De Geer selects different leaf position along the apple shoots (Whitaker et al, 2006). Trumble (1982) showed that the best sampling procedure of aphids are different in broccoli. Yarahmadi, Soleyman Nejadian, Mohisseni (2008) reported that the wheat aphids (*Sitobion avenae* Rodani, *Schizaphis graminum* Fabricus and *Diuraphis noxia* Mordviko) choose different parts of wheat during their feeding activity and the behaviors affected their suitable sample universes.

The yellow sticky and yellow basin traps are only suitable for population monitoring of *T. aurantii* on Satsuma mandarin. For other aphid and host plants, the traps are not appropriate for estimating population. In contrast, Marroquin et al (2004) used yellow sticky and yellow basin traps for monitoring citrus aphids in various citrus orchards in Spain. Our result is in line with Han, Han, Zhang, & Byers (2012) who showed that *T. aurantii* attracted to yellow sticky traps. Straw et al. (2011) demonstrated that the trap color significantly affected the capture of aphid alate. Therefore, other color of sticky or basin trap may be efficient for monitoring of the aphids population on Satsuma mandarin or Thomson navel orange.

In conclusion, the best sampling procedures of *A. spiraecola*, *A. gossypii* and *T. aurantii* were significantly influenced by aphid or host plant species. Also, yellow sticky and yellow basin traps are nearby not suitable for population monitoring of the aphids on Satsuma mandarin and Thomson navel orange.

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