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Distribution of population of immature stages of common pistachio psyllid, *Agonoscena pistaciae* within the tree and development of sampling strategy

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Abstract
The distribution of population of egg and different nymph stages of common pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) within the pistachio tree canopy were investigated in Rafsanjan during 2013 and 2014. Also, in this study, the preference of pest to lay egg and activity on young, mature and old leaves, light and dark green leaves and leaves available in shade and sun were evaluated. The results indicate that common pistachio psyllid infestation was higher significantly in the upper and middle parts of the tree canopy compared with the lower parts. Means population density of egg and nymph were higher significantly on mature, dark green leaves and leaves in sun in comparison to young, light green leaves and leaves available in shade. Therefore, the results of this study indicated that common pistachio psyllid population is not randomly distributed in various sections and leaves of the pistachio tree.

Keywords: *Agonoscena pistaciae*, pistachio tree canopy, leaf age, leaf color, leaf position

1. Introduction

The pistachio, *Pistacia vera* L. (Sapindales: Anacardiaceae), is one of the most important horticultural products in Iran [8]. The common pistachio psyllid (CPP), *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae), is a key pest of pistachio trees throughout the pistachio producing regions of Iran. Both nymphs and adults suck sap from leaves and causes significant damage to pistachio trees [8]. This pest causes defoliation, falling flower buds and eventually stops tree growth and leads to the loss of crops and produces large amounts of honeydew [8, 23]. The development of a sampling program to monitor insect populations is a fundamental tool for integrated pest management (IPM) [26]. As IPM is an ecology-based approach to pest management that relies on current information about the status of the pest and the crop, a sampling program is critical for decision-making tactics [20]. Data on dispersion of pest populations is an important aspect of population biology because it is a result of the interaction between individuals of the species and their habitat [25]. Knowledge of this dispersion allows a better understanding of the relationship between an insect and its environment and provides basic information for interpreting spatial dynamics, designing efficient sampling programs for population estimation, and pest management [7, 13, 25, 30], Although many aspects of CPP biology have been investigated [18] it was realized that detailed population studies, which will comprise the simultaneous assessment of the main population parameters, are necessary to provide the basis for the development of a pest management system utilizing ecological criteria. The knowledge of the distribution of the infestation of CPP within the tree, as in most insects, is considered necessary for the development of optimum sampling plans [28] but such knowledge is limited [29]. On the other hand, CPP infestation is very high during certain periods of the year (e.g. late spring-early summer) and it is important to keep the number of samples to a minimum. Therefore, the pattern of sampling (random or stratified) is critical to minimize variance [28]. Thus, the objectives of the present study were to determine preferred canopy strata of CPP in pistachio trees canopy. Also, to evaluate preference of the pest on leaves attention to age, color and position (in sun or shade). These data will be a necessary first step in the development of an IPM program against the CPP where there is a need quickly to estimate the population density of this pest, to design different control method and generally the study various aspects of this pest.

2. Materials and Methods

2.1. *Study site*

The trials were conducted in a pesticide free pistachio orchard cv. 'Akbari' (20-year-old trees) in Rafsanjan, Iran during summer 2013-2014.

2.2. *Sampling*

Ten pistachio trees in center of rows selected randomly for sampling. The canopy of each tree was partitioned into three strata upper, middle and lower. For each strata, ten trees and from each tree, four leaves from each side (40 leaves total) each tree were picked randomly. The number of eggs and nymphs of CPP on upper and lower sides of apical leaflet were counted and recorded. Also, at this study, evaluated the response of CPP to leaf age (old, mature and young leaves), leaf color (dark and light green leaves) and leaf position (in shade and sun). Forty replicates for each case were collected from ten trees that were selected randomly. The number of eggs and nymphs on upper and lower sides of apical leaflet were counted and recorded. Sampling of leaf position was carried out at mid-day and Lux meter LX-101 was used to measure light intensity.

2.3. *Statistical analysis*

The analysis of data was performed using SPSS (version16, SPSS Inc) software and the comparison of means by Tukey's test for the effect of tree canopy height and leaflet age and t-test for the effect leaflet color and position (in shade and sun) ($P < 0.05$).

3. Results

3.1. *The effect of heights of trees canopy on population density of egg and nymph stages*

Fig. 1 shows population distribution of egg and nymph of CPP within the tree in relation to Levels (upper, middle and lower part of the tree canopy) in 2013 and 2014. There were significant differences in egg population density on upper, middle and lower part in 2013 ($F = 38.99$; df = 2, 117; $P = 0.0001$) and 2014 ($F = 81.77$; df = 2, 117; $P = 0.0001$ (Fig. 1). The results showed that density of eggs and nymphs were higher on upper and middle part (Fig. 1). So that, egg population density mean on upper, middle and lower part recorded 64.95 ± 8.73, 36.64 ± 5.79 and 17.07 ± 2.38 per leaflet respectively, in 2013. But, in 2014 egg population density mean obtained 44.45 ± 3.77, 38.77 ± 4.13 and 19.62 ± 1.76 per leaflet respectively (Fig. 1). Significantly ($P < 0.05$) high numbers of immature stages were found in the upper canopy compared to the lower canopy. Nymph population density mean on upper, middle and lower part obtained 98.17 ± 8.41, 86.82 ± 6.67 and 25.70 ± 2.31 per leaflet respectively, in 2013 (Fig. 1). But, in 2014 nymph population density mean decrease and recorded 87.82 ± 4.72, 79.7 ± 4.59 and 19.62 ± 1.76 per leaflet respectively (Fig. 1).

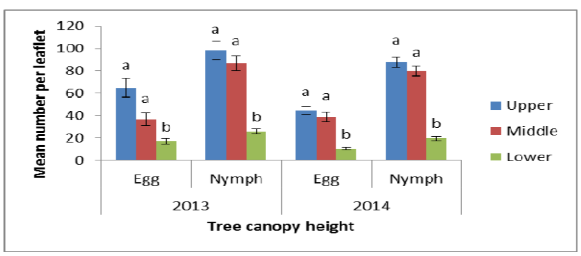

Fig 1: Mean (±SE) egg and nymph density in upper, middle and lower parts of the pistachio tree canopy in 2013 and 2014. Means comparison consistent with a Tukey's test. Different letters indicate significant differences ($P < 0.05$).

3.2. *The effect of leaf age on egg and nymph population density*

Results indicated that there were significant differences in egg population density on young, mature and old leaves in 2013 ($F = 59.95$; df = 2, 117; $P = 0.0001$) and 2014 ($F = 65.95$; df = 2, 117; $P = 0.0001$). Egg population density mean on young, mature and old leaves recorded 6.82 ± 1.23, 42 ± 4.54 and 3.32 ± 0.85 per leaflet respectively, in 2013 (Fig. 2). But, mean the number of egg obtained 8.70 ± 1.37, 61.42 ± 5.77 and 4.50 ± 1.30 per leaflet respectively, in 2014 and showed significant differences (Fig. 2). As, Fig. 2 shown, there were significant differences in nymph population density on young, mature and old leaves in 2013 ($F = 215.20$; df = 2, 117; $P = 0.0001$) and 2014 ($F = 167.63$; df = 2, 117; $P = 0.0001$). So that, nymph population densities on young, mature and old leaves recorded 15.42 ± 2.22, 101.38 ± 5.59 and 8.45 ± 1.05 per leaflet respectively, in 2013 (Fig. 2). The mean population density of nymph decreased in 2014 recorded 13.55 ± 1.89, 90.75 ± 5.84 and 7.37 ± 0.95 per leaflet on young, mature and old leaves respectively (Fig. 2). Generally, the highest of egg and nymph population density recorded on mature leaves and show significant differences with young and old leaves. But, mean of egg and nymph density on young and old leaves showed no significant differences and were categorized in a group (Fig. 2).

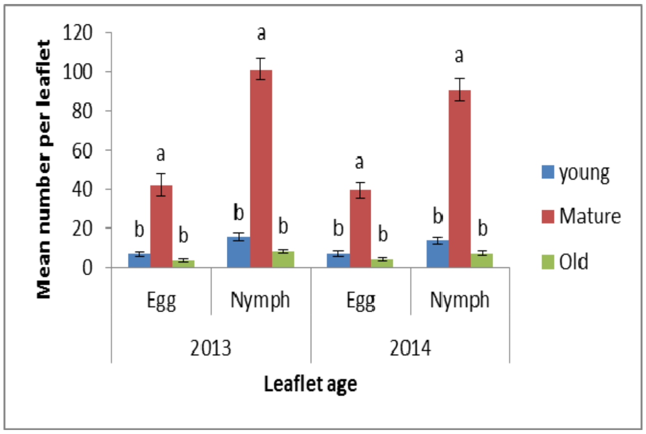

Fig 2: Means (±SE) egg and nymph density on young, mature and old leaves in 2013 and 2014. Means comparison consistent with a Tukey's test. Different letters indicate significant differences ($P < 0.05$).

3.3. *The effect of leaf color on egg and nymph population density*

Fig. 3 shows the distribution of CPP infestation on dark and light green leaves. There were significant differences in egg and nymph density on dark and light green leaves (Fig. 3). Results indicated that egg and nymph population density were significantly higher on dark green leaves than light green (Fig. 3). Mean the number of egg on dark and light green leaves were recorded 36.40 ± 3.04 and 5.45 ± 0.87 per leaflet respectively, in 2013 ($t = 9.75$; df = 45.34; $P = 0.0001$). Also, in 2014, mean the number of egg on dark and light green leaves (45.12 ± 3.97 and 3.07 ± 0.44 per leaflet respectively) showed significant differences ($t = 10.50$; df = 39.97; $P = 0.0001$). As Fig. 3 shown mean the number of nymph on dark and light green leaves recorded 82.00 ± 4.30 and 16.60 ± 2.42 per leaflet respectively, in 2013 ($t = 13.24$; df = 61.49; $P = 0.0001$) and 88.07 ± 5.66 and 12.32 ± 1.56 per leaflet in 2014 ($t = 12.89$; df = 44.96; $P = 0.0001$). Mean the number of nymph on dark and light green leaves obtained lower in 2014 (Fig. 3).

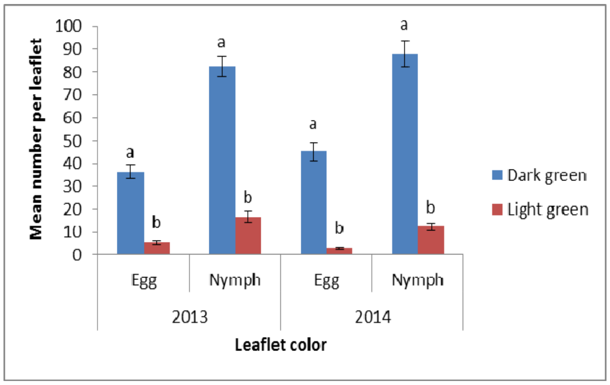

Fig 3: Means (±SE) egg and nymph density on dark and light green leaves in 2013 and 2014. Means comparison consistent with t –tests. Different letters indicate significant differences (P < 0.05)

3.4. *The effect of leaf position (sun and shade) on egg and nymph population density*

Results indicated that there were significant differences in egg and nymph population density on leaves available in sun and shade. So that, egg population density mean on leaves available in sun and shade obtained 51.80 ± 3.33 and 11.32 ± 1.17 per leaflet (t = 11.44; df = 48.45; P = 0.0001) respectively, in 2013. But, mean the number of nymph on leaves available in sun and shade recorded 157.50 ± 8.46 and 22.57 ± 2.55 per leaflet (t = 15.26; df = 46.04; P = 0.0001) respectively, in 2013. Whereas, in 2014, egg population density on leaves available in sun and shade obtained 45.32 ± 3.51 and 8.67 ± 0.90 per leaflet (t = 10.09; df = 44.19; P = 0.0001) respectively and 135.50 ± 7.18 and 24.45 ± 2.82 per leaflet (t = 14.37; df = 50.78; P = 0.0001) respectively, for nymph population density.

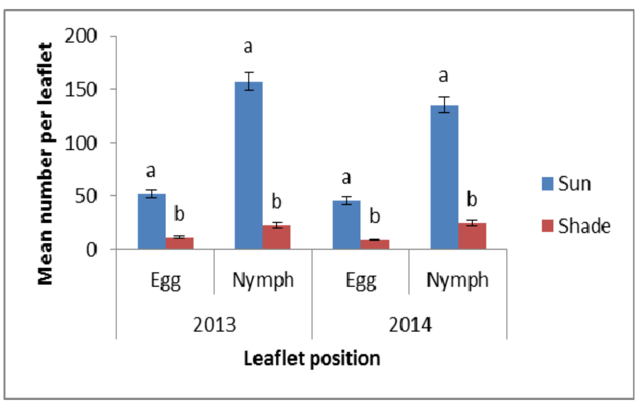

Fig 4: Means (±SE) egg and nymph density on leaves in sun and shade in 2013 and 2014. Means comparison consistent with t –tests. Different letters indicate significant differences (P < 0.05).

4. Discussion

4.1. *The effect of pistachio tree canopy height on population density of egg and nymph stages*

For the development of a sampling plan, choosing an appropriate sample unit is important [26]. These sampling plans are is necessary for integrated pest management program for this pest. Dispersion data allow a better understanding of the relationship between an insect and its environment and provide basic knowledge for interpreting spatial dynamics and designing efficient sampling programs [31]. The analysis of the results obtained in this study indicated that CPP infestation is not randomly distributed in relation various sections of the tree. The preferred oviposition sites of the females of CPP are in the upper parts of the canopy probably because temperature requirements are better met in these sections. Of course, the population densities on the upper and middle sections of the canopy tree show no significant differences. The lower density of eggs and nymphs on low strata of tree were lower because, probably this leaves had lower nutrient quality and ecological conditions are unsuitable to complete life cycle. The high population of eggs and nymphs on the upper canopy in the present study is attributed to influence of other physical factors such as exposure to sunlight, temperature and relative humidity. Butler and Trumble [3] reported the most efficient sampling unit for the potato psyllid in potatoes involved examining the edges of the fields and sampling the underside of leaves in the middle or top of the plant. Other psyllid species such as *Diaphorina citri* Kuwayama and *Trioza erytreae* (Del Guercio) also exhibit aggregated spatial patterns [24, 31]. Generally, the higher aggregation of CPP on upper and middle strata of pistachio canopy could be related to either feature of this psyllid (such as behavior and reproductive biology), or to some heterogeneity of the environment (such as microclimate, preferred part of plant, and natural enemies) [28]. The similar observations were also reported by [32] about the citrus psylla (*Trioza erytreae*). Soemargono *et al*. [29] investigated distribution of *D. citri* on citrus and orange jasmine and reported distribution between the upper and lower half of the canopy was significantly different where the upper canopy harbored more psyllids than the lower half. Also, Stratopoulou and Kapatos [31] was evaluated distribution of population of pear psylla on upper and lower part of pear tree and showed the higher density this pest on upper part compared to lower part.

4.2. *The effect of leaf age on egg and nymph population density*

There is essentially no information on the chemical and physical stimuli that influence oviposition. However, once a host has been selected, oviposition site location and preference can be influenced by leaf age and surface texture cues [10, 11]. Even no preferred oviposition sites on a leaf could be made suitable by introducing an artificial mid-vein [10]. Variations in density of immature stages on pistachio tree leaves in regard to age show that highest levels occur in mature leaves, likely due to the leaf nutrient resorption mechanism of senescing leaves [2, 23] and leaves color. The nutrient resorption mechanism is considered one of the most important plant nutrient conservation mechanisms [12, 34, 35]. Foliar nutrient concentrations remain relatively constant from the time of full leaf expansion to the beginning of senescence and then decrease rapidly as foliar nutrients are resorbed prior to abscission [9]. N, P, and K are mobile nutrients that are easily withdrawn from senescing tissues, and K is known for leaching [6, 16, 21]. These data indicate that lower nutrient quality of old leaves compared to mature leaves that related to high nutritive resorption during leaf senescence. The present study indicated that probably mature leaves are essential for CPP to enter into the reproductive phase and complete its life cycle. Probably, the low density of immature stages on old leaves compared with mature leaves exhibit these leaves are unsuitable to complete life cycle of this pest and cause greater mortality and generally is a failure. The observed high number of eggs and nymphs on mature leaves than young leaves may be attributed to leaves color because; young leaves are light green and female psyllid reluctant to lay egg on light green leaves that were described in next section. Of course, Yang *et al.* [31] showed numbers of tracks in citrus leaves by feeding *D. citri* declined markedly with increasing leaf maturity.

4.3. *The effect of leaf color on egg and nymph population density*

Results indicated that significantly more egg and nymph were observed on dark green leaves than light green leaves that suggests the psyllid orientation to this color is the result of a positive attraction. The results demonstrate that CPP adults are highly responsive dark green leaves. Therefore lay higher egg on these leaves. Kring [16] found that *Aphis nasturtii* was more attracted to full yellow than to lighter tints of yellow. Prokopy and Owen (1983) reported that within the visible spectrum of diurnal insects (300-650 nm) the surface of green leaves reflected maximum energy between 500- 600 nm with a peak at 550 nm. Therefore, if CPP detects pistachio foliage at least in part on the basis of color, they should be responsive to energy reflected in the 500-600 nm region of the spectrum. Mensah and Madden [21] evaluated color preferences of psylla *Ctenarytaina thysanura* and showed that green, blue and yellow all reflected considerable energy in the 500-560 nm range, but yellow reflected most. These researchers suggested that the greater intensity of reflection from yellow hue in the general part of the spectrum where green leaves reflect most light i.e. 500-600 nm could be the basis for the great attraction of *C. thysanura* adults to yellow. A considerable number of other insect species also have been reported to be attracted to yellow. These include *Psylla pyricola* [1], as well as other homopterans [14]. Also, it has been proved that the greenhouse whitefly, *Trialeurodes vaporariorum* [4] and asparagus miner, *Ophiomyia simplex* [5] respond positively to yellow.

4.4. *The effect of leaf position on egg and nymph population density*

The result indicated that there were significant differences in population density of egg and nymph on leaves available in sun than shade. The population density of egg and nymph were higher on leaves available in sun. Because, as were above-mentioned (section 4.1) temperature requirements are better in sections and leaves available in sun. As a result, ecological conditions are suitable to complete life cycle at leaves available in sun.

5. Conclusion

The results and the conclusions from this study provide the basis for the development of a sampling program for the study of CPP populations that will minimize systematic errors [17] which arise from a random sampling in a no randomly distributed population. It is suggested, therefore, that samples for the estimation of CPP population of immature stages must be taken from the middle of the tree canopy that expresses the general trend of the distribution of population in regard to height. Also, sampling must be carried out from middle leaves of pistachio shoots. Generally, the sampling must be carried out from leaves having the same condition and character. So, sampling plans based on dispersion descriptions of this pest reduce sampling effort and minimize variation of sampling precision.

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