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Attraction of *Agonoscena pistaciae* (Hemiptera: Aphalaridae) to male or female psyllid in field condition

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Abstract

The pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae), is the key pest of pistachio trees in Iran. The objective of this study was to field test, whether female or male of *A. pistaciae* attracted male or female pistachio psyllid in the fields. Sticky traps were used to assess whether traps with live male or female pistachio psyllid attract conspecific psyllid in the field. Traps contain 20 live females, traps contain 20 live males, or traps without male or female (control). We compared mean number of male or female psyllid to choose control, male or female baited traps. Results in year 2014 showed that the mean number of male attracted to control, male and female traps was 20.87±2.88, 24.20±3.72 and 25.80±3.38 psyllid, respectively. The mean number of female attracted to control, male and female traps was 20.93±3.11, 31.40±3.68 and 19.60±2.80 psyllid, respectively. Results in year 2015 showed that The mean number of male attracted to control, male and female traps was 54.13±9.11, 73.44±16.77 and 51.06±7.42 psyllid, respectively. The mean number of female attracted to control, male and female traps was 57.31±8.50, 93.13±19.79 and 54.06±10.23 psyllid, respectively. Results in year 2016 showed that the mean number of males attracted to control, male and female traps were 31.75±3.42, 29.00±4.42 and 45.00±3.16 psyllid, respectively. The mean number of female attracted to control, male and female traps was 31.44±3.45, 33.94±5.65.79 and 41.88±3 psyllid, respectively. This results can be useful for the development of more controlling methods for this pest.

Key words: *Agonoscena pistaciae*, sex pheromone, attraction

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Introduction

Pistachio, *Pistacia vera* L., is one of the most important horticultural products in Iran (Javadian and Farzane 2005). This product is important form economic, social and environmental aspects (Esmailpor *et al*. 2011). The pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae) is a major pest of pistachio trees throughout pistachio – producing regions in Iran (Hassani *et al*. 2009a, Hassani 2011). Both nymphs and adults suck sap from leaves and produce large amounts of honeydew. Direct feeding causes reduced plant vigor, defoliation, stunting, poor yield and bud drop (Hassani *et al*. 2009a; Mehnejad 2010, 2014). This pest has six complete and one incomplete generation per year (Hassani *et al*. 2009b). This pest control by applying chemical insecticides several times each year (Mehrnejad 2010). Integrated pest management (IPM) programs emphasize the combination of tactics, to maintain pest populations below economic thresholds. Although combining tactics may provide better long-term sustainable pest suppression than one tactic alone. Insecticides can disrupt natural enemies through lethal and sub-lethal means causing pest resurgence or secondary pest outbreaks (Roubos 2014). Pheromones and other semiochemicals instead can be implemented for sustainable area-wide management and will thus improve food security for a growing population. Future applications of pheromones and other semiochemicals depend on the availability of odorants that enable efficient manipulation of mate and host-finding behavior in insects and other animals. Continued, goal-oriented research aimed at the identification of behaviorally relevant odorants will continue to bring forth novel insect control methods that contribute increasingly to food and environmental security (Witzgall 2010). Mate finding and courtship involve complex interactions that require close coordination between individuals of the opposite gender. Several studies have shown that semiochemicals play a role in psyllid mate finding (Lubanga *et al*. 2014). Brown *et al*. (2009) tested whether female *Cacopsylla pyricola* (Förster) attracted male winterform and summerform of pear psylla in the field. In both trials, there was a significant preference by males for the female-baited traps compared with unbaited traps. Laboratory studies have shown that volatile cues are used by males of some Psyllidae in locating females (Soroker *et al*. 2004), including by males of *C. pyricola* (Horton and Landolt 2007; Horton *et al*. 2007; Horton *et al*. 2008; Guedot *et al*. 2009). Female-produce volatile sex attractant pheromone in *Diaphorinia citri* Kuwayama (Wenninger *et al*. 2008). In laboratory olfactometer assays, potato psyllid males were attracted to odorants emitted from live females (Guedot *et al*. 2010).

There was no information about the role of mate locating behavior in affecting movement by the pistachio psyllid. The objective of this study was to test, whether female or male of *A. pistaciae* attracted conspecific psyllid in the fields.

Material and Methods

Insect Collection

The experiments were done in a pistachio orchard located in Rafsanjan, Kerman province, and the pistachio height of trees were 2 m. During the experiment do not used any insecticide in the orchard. Adult's male and female sumerform psyllid were collected form the selected orchard by a 40 * 40 cm beat tray trap by beating on pistachio branches. Identify of Males and females were done by sex and aspirated into vials by stereomicroscope. Males and females separated by examining the posterior end of the abdomen.

Trap design

Sticky traps were used to assess whether traps with live male or female pistachio psyllid attract conspecific psyllid in the field. Sticky traps design based on Brown *et al*. (2009). Sticky traps consisted of an inner cage that held the attractant (live female or male psyllid) and an outer screen designed (white-colored) to capture psyllid that came into contact with the trap. Traps contain 20 live females, traps contain 20 live males, or traps without male or female (control).

The experiments were done during 3 years. In 2014, each of three treatments done 5 times with 3 replications, and in 2015 and 2016, 4 times with 4 replications. The traps were placed at 1.5 height of tree canopy. Traps were left in the field for 4 days and after that, traps transferred to laboratory and the trapped males and females were counted in each trap by stereomicroscope.

Data Analysis

We compared the mean number of male and female psyllid attracted to control or traps contain male or female. Data were analyzed using by ANOVA using SAS version 9.1. And the means were separated by Tukey test.

Results

Experiments in 2014

Results in year 2014 showed that there was not significant among the number of male attracted to treatments (Fig. 1; Table 1). The mean number of male attracted to control, male and female traps was 20.87±2.88, 24.20±3.72 and 25.80±3.38 adult male psyllid. Whereas there was significant among the number of female attracted to treatments and the females were more attracted to traps baited with male than on female or unbaited traps (Fig. 1; Table 1). The mean number of female attracted to control, male and female traps was 20.93±3.11, 31.40±3.68 and 19.60±2.80 adult female psyllid.

Fig. 1. Mean number of pistachio psyllid attracted to treatments (control, male and female) in year 2014. Means with different letters are significantly different (Tukey test, $P < 0.05$).

Table 1. Analysis of variance effect of attracted male and female to male, female or unbaited traps in year 2014

Experiments in 2015

Results in year 2015 showed that there was not significant among the number of male attracted to treatments. However male were more attracted to the traps baited with male than female and unbaited traps (Fig. 2; Table 2). The mean number of male attracted to control, male and female traps was 54.13±9.11, 73.44±16.77 and 51.06±7.42 adult male psyllid. But there was significant among the number of female attracted to male and female. The females were more attracted to traps baited with male than on female (Fig. 1; Table 1). And there was not significant between the number of female attracted to female and unbaited traps. The mean number of female attracted to control, male and female traps was 57.31±8.50, 93.13±19.79 and 54.06±10.23 adult female psyllid.

Fig. 2. Mean number of pistachio psyllid attracted to treatments (control, male and female) in year 2015. Means with different letters are significantly different (Tukey test, $P < 0.05$).

Table 2. Analysis of variance effect of attracted male and female to male, female or unbaited traps in year 2015

Experiments in 2016

Results in year 2016 showed that there was significant difference among the number of male attracted to treatments. The mean number of male were significantly more on female than male and unbaited traps (Fig. 3; Table 3). The mean number of males attracted to control, male and female traps were 31.75±3.42, 29.00±4.42 and 45.00±3.16 adult male psyllid. There was significant among the number of female attracted to treatments. But there was not significant difference between the number of female attracted to male and female. However the females were more attracted to traps baited with female than male and unbaited traps (Fig. 3; Table 3).

The mean number of female attracted to control, male and female traps was 31.44±3.45, 33.94±5.65.79 and 41.88±3.21 adult female psyllid.

Fig. 3. Mean number of pistachio psyllid to attracted to treatments (control, male and female) in year 2016. Means with different letters are significantly different (Tukey test, $P < 0.05$).

Table 3. Analysis of variance effect of attracted male and female to male, female or unbaited traps in year 2016

Discusion

Three types of cues are used in the Psyllids as means for locating mates: visual, acoustic (including substrate vibrations), and olfactory (Brown *et al*. 2009; Liao and Yang 2015). Mate finding and courtship involve complex interactions that require close coordination between individuals of the opposite gender (Lubanga *et al*. 2014).

The results showed that in years 2014 and 2015 there was not significant preference between the numbers of male attracted to treatments (baited or unbaited traps). In year 2014 the number of male attracted to male was more than female. This results may be because of aggregation pheromone in adult male of pistachio psyllid. The results showed that potato psyllid males are attracted to both female and male-produced volatile chemicals might be used as male aggregation pheromone in potato psyllid (Guedot *et al*. 2010). Another reason can be more population density in this year. Because pheromones are increasingly efficient at low population densities (Witzgall 2010). But there was significant difference between the number of male attracted to male and female in year 2016. The males were significantly more on traps baited with females than on traps baited with male or unbaited traps (control). The field trials suggested that *C. pyricola* males actively searched for females, possibly using volatiles emitted by the females (Brown *et al*. 2009). In the asian citrus psyllid, *D. citri* stimulatory cuticular hydrocarbons act as sex pheromone attractants. Male psyllids locate aggregations of females using those olfactory cues, as well as vibrational communication on the plant surface (Stockton 2017). The results of Brown *et al*. (2009) showed that winterform *C. pyricolla* males were significantly more on traps baited with females than on males and unbaited traps. Laboratory and field studies have shown that volatile cues are used by males of *C. pyricola* (Horton and Landolt 2007; Horton *et al*. 2007). Female-produce volatile sex attractant pheromone in *Diaphorinia citri* (Wenninger *et al*. 2008). In laboratory olfactometer assays, the potato psyllid, *Bactericera cockerelli* (Šulc) males were attracted to odorants emitted from live females. Males were also attracted to volatile chemicals from males, providing the first example of male-male attraction in the Psylloidea. (Guedot *et al*. 2010). Potato psyllid females and males were attracted to female-produced volatile chemicals, which may be evidence for the existence of a female-specific sex attractant for males (Guedot *et al*. 2010).

The number of males and females attracted to unbaited traps were almost the same during 3 years. The results of Brown *et al*. (2009) showed that both male and female winterform *C. pyricolla* were consistently caught on control traps in all experiments. The results of years 2014 and 2015 showed that there was significant preference between the number of female attracted to male and female. The females were significantly more on traps baited with males than on traps baited with female. But, there was not significant preference between the number of females attracted to male and female in year 2016.

The color of traps, environmentally conditions, and use of mated or unmated male or females probably were effected in males attraction to females. May be, this species use visual or acoustic cues to locate or accept mates. There is evidence that a number of other psyllid species use acoustic cues or substrate vibrations to locate or accept mates (Tishechkin 2007; Percy *et al*. 2006). The vibrational signals of psylloids were for mating and species recognition (Liao and Yang 2015). Wenninger *et al*. (2009) found no evidence to support the hypothesis that male calling behavior is elevated by the detection of female and/or host plant odors. However, latency to calling was significantly shorter for males observed in the presence of clean air.

Many psyllid species rely on vibrational signals for mate finding and mate assessment during courtship. This apparent disproportional reliance on vibrational rather than semiochemical signals suggests that vibrational signals have been more influential in sexual selection in psyllids (Lubanga *et al*. 2014). The initial call and active search are usually made by the male. Females usually stay stationary and respond passively. Signals from males include two sections: a series of chirps followed by a long trill, whereas those from females include a long series of repeated chirps. The results of a discriminant analysis of acoustics showed that three species of *Cacopsylla* were distinguishable from acoustic characteristics. (Liao and Yang 2015). Males and females both respond to signals produced by either sex (Mankin *et al*. 2013). Eben *et al*. (2014) found potentially sound producing stridulatory organs in male and female individuals of *C. pyri* (Eben *et al*. 2014). The compounds identified thus far, namely 13-methylheptacosane (from *Cacopsylla pyricola*) and dodecanoic acid (from *Diaphorina citri*), seem to have short range activity or no activity under field conditions (Lubanga *et al*. 2014). However, even if a sex pheromone is identified, for a species like *D. citri* in which vibrational communication is an important part of mate location, the use of pheromones for monitoring and/or management may be challenging. For example, possible future efforts to reduce mating rates by sex pheromone based mating disruption or attract-and-kill might be compromised if the sexes are able to find each other using primarily acoustic cues (Wenninger *et al*. 2009). Additional studies is need for the relative roles of acoustic and chemical cues in mate location in this species as well as how mate location might be influenced by interactions among psyllids and their host plants.

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Table 1. Analysis of variance effect of attracted male and female to male, female or unbaited traps in year 2014

Effect	df	Males		Females	
		F	*P*	*F*	*P*
Date	4	7.29	0.0009	4.08	0.0141
Block	10	0.78	0.6480	0.38	0.9425
Treatment	2	0.93	0.4102	5.25	0.0147
Treatment*date	8	1.52	0.2121	1.82	0.1315
Error					

Table 2. Analysis of variance effect of attracted male and female to male, female or unbaited traps in year 2015

Effect	df	Males		Females	
		F	*P*	*F*	*P*
Date	3	3.98	0.0196	7.08	0.0014
Block	12	1.20	0.3389	1.30	0.2790
Treatment	2	1.45	0.2552	4.34	0.0246
Treatment*date	6	1.92	0.1184	2.98	0.0254
Error	24				

Table 3. Analysis of variance effect of attracted male and female to male, female or unbaited traps in year 2016

effect	df	Males		Females	
		F	*P*	*F*	*P*
Date	3	12.01	<.0001	16.73	<.0001
Block	12	0.71	0.7254	0.79	0.6569
Treatment	2	9.39	0.0010	4.15	0.0282
Treatment*date	6	1.77	0.1483	4.96	0.0020
Error	24				

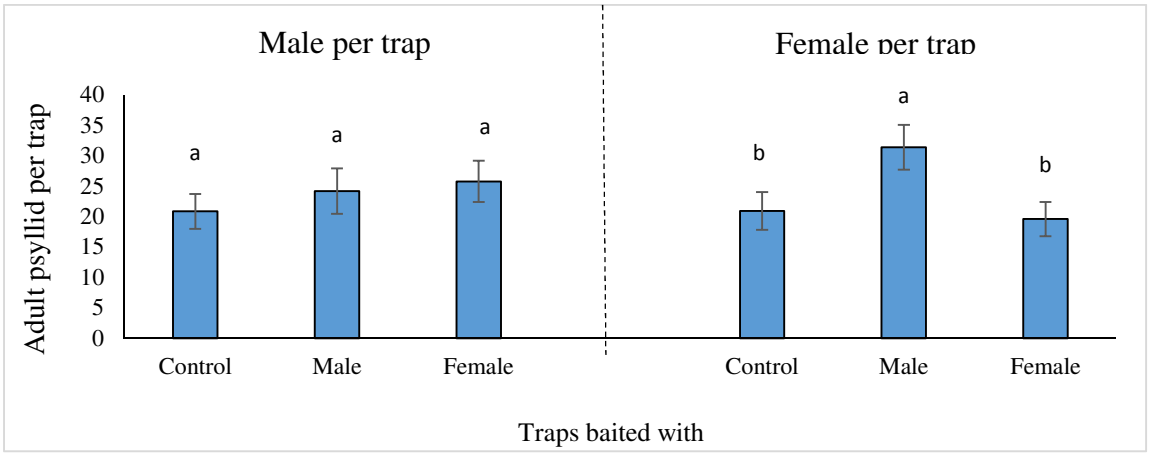

Fig. 1. Mean number of pistachio psyllid attracted to treatments (control, male and female) in year 2014. Means with different letters are significantly different (Tukey test, P < 0.05).

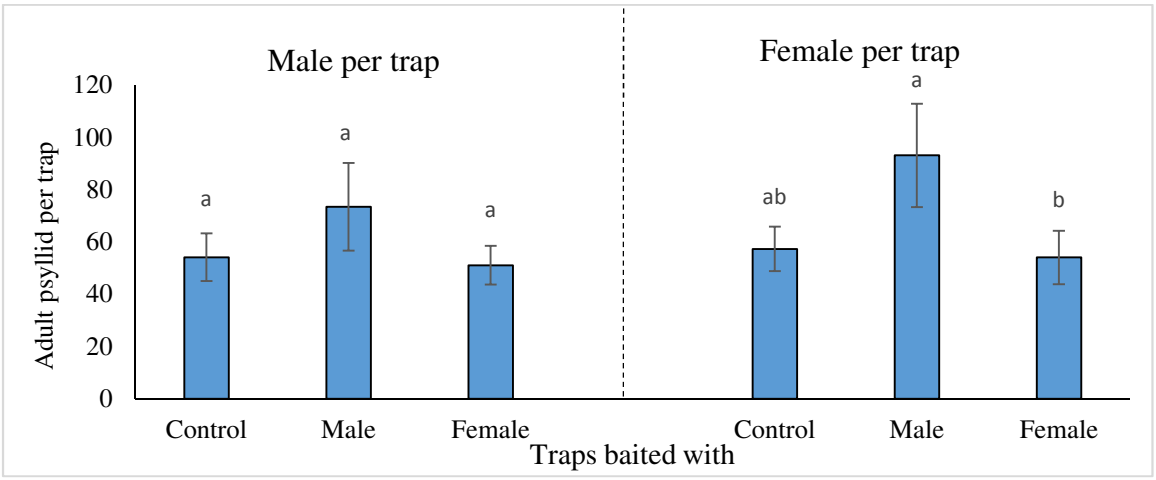

Fig. 2. Mean number of pistachio psyllid attracted to treatments (control, male and female) in year 2015. Means with different letters are significantly different (Tukey test, P < 0.05).

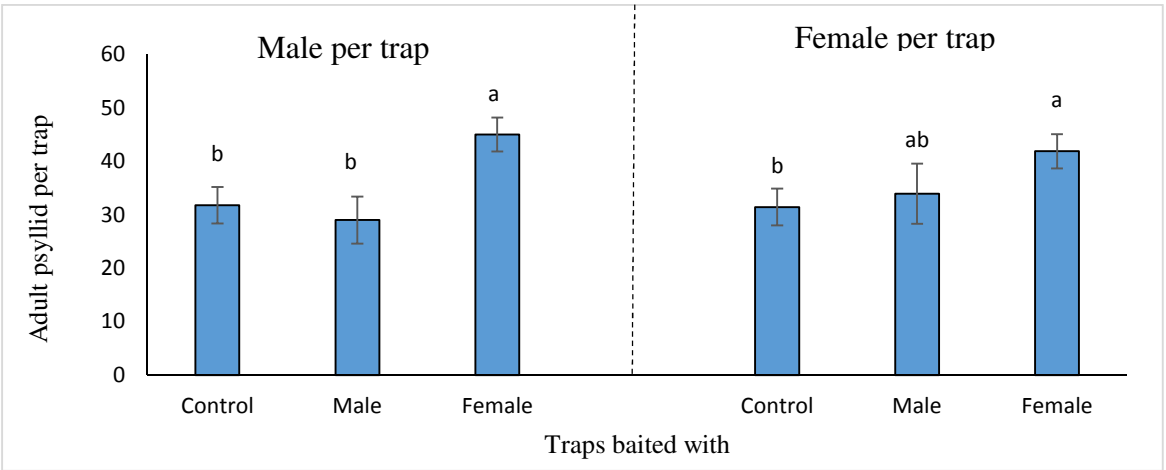

Fig. 3. Mean number of pistachio psyllid to attracted to treatments (control, male and female) in year 2016. Means with different letters are significantly different (Tukey test, P < 0.05).

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جلب شدن حشرات نر یا ماده پسیل پسته *Agonoscena pistaciae* (Hemiptera: Aphalaridae) به حشرات نر یا ماده در شرایط باغ

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چکیده

پسیل معمولی پسته *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Aphalaridae) آفت کلیدی درختان پسته در بیشتر مناطق پسته‌کاری ایران است. هدف از این پژوهش بررسی امکان وجود ترکیبات شیمیایی جلب‌کننده جنس مخالف در حشرات نر و ماده پسیل پسته در شرایط طبیعی باغ می‌باشد. از تله‌های چسبنده حاوی حشرات نر یا ماده و کنترل (بدون حشره نر یا ماده) برای ارزیابی میزان جلب‌شدن حشرات نر یا ماده به جنس مخالف خود استفاده شد. تله‌های چسبنده حاوی ۲۰ حشره نر یا ۲۰ حشره ماده و تله‌های کنترل (بدون حشره نر یا ماده) بودند. نتایج آزمایش‌های باغی در سال ۱۳۹۳ نشان داد، متوسط تعداد حشرات نر جلب شده به تله‌های کنترل، حشرات نر و ماده به‌ترتیب ۲/۸۸±۲۰/۸۷، ۳/۷۲±۲٤/۲۰ و ۳/۳۸±۲۵/۸۰ به‌دست آمد. متوسط تعداد حشرات ماده جلب شده به تله‌های کنترل، حشرات نر و ماده به‌ترتیب ۳/۱۱±۲۰/۹۳، ۳/٦۸±۳۱/٤۰ و ۲/۸۰±۱۹/٦۰ به‌دست آمد. نتایج سال ۱۳۹٤ نشان داد، متوسط تعداد حشرات نر جلب شده به تله‌های کنترل، حشرات نر و ماده به‌ترتیب ۷/٤۲±۵۱/۰٦، ۱٦/۷۷±۷۳/٤٤ و ۹/۱۱±۵٤/۱۳ به‌دست آمد. متوسط تعداد حشرات ماده جلب شده به تله‌های کنترل، حشرات نر و ماده به‌ترتیب ۱۰/۲۳±۵٤/۰٦، ۱۹/۷۹±۹۳/۱۳ و ۸/۵۰±۵۷/۳۱ به‌دست آمد. نتایج سال ۱۳۹۵ نشان داد متوسط تعداد حشرات نر جلب شده به تله‌های کنترل، حشرات نر و ماده به‌ترتیب ۳/۱٦±٤۵/۰۰، ٤/٤۲±۲۹/۰۰ و ۳/٤۲±۳۱/۷۵ به‌دست آمد. متوسط تعداد حشرات ماده جلب شده به تله‌های کنترل، حشرات نر و ماده به‌ترتیب ۳/۱۱±۲۰/۹۳، ۳/٦۸±۳۱/٤۰ و ۲/۸۰±۱۹/٦۰ به‌دست آمد. نتایج حاصل از این پژوهش می‌تواند در توسعه برنامه‌های مدیریت این آفت موثر باشد.

واژه‌های کلیدی: پسیل پسته، فرمون جنسی، جلب‌کنندگی

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