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**INITIAL STUDY OF REARING AND RELEASE OF *Anthocoris minki* Dohrn (HEMIPTERA: ANTHOCORIDAE) FOR BIOLOGICAL CONTROL OF *Agonoscena pistaciae* Burckhardt and Lauterer (HEMIPTERA: PSYLLIDAE) IN PISTACHIO ORCHARDS**

**ABSTRACT**

The pistachio psyllid, *Agonoscena pistaciae* Burckhardt & Lauterer 1989 (Hemiptera: Psyllidae), is an important pest of pistachio in Şanlıurfa, Turkey. In this study, laboratory reared *Anthocoris minki* Dohrn (Hemiptera: Anthocoridae) was released for biological control of pistachio psylla in pistachio orchards. Frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were utilized as prey of rearing of *A. minki* in the laboratory. The experiment was carried out between May and November in 2006. In the second week of July, *A. minki* caused reduction the density of pistachio psyllas' from 175.6 to 31.3 nymph/composite leaf. However, in the beginning of August, due to infestations from a neighboring orchard, population density of the pistachio psylla increased to 193.4 nymph/composite leaf. Therefore, in the second week of July, an additional release of *A. minki* was performed. Towards the end of August the pistachio psylla population density was reduced to the economic injury threshold level. In the control orchard, where no *A. minki* was released, pistachio psylla reached the density of 458 nymph/composite leaf in spite of insecticide use. The results of this initial study indicated that this predator species may be effective for biological control of *A. pistaciae*.

**Keywords:** *Anthocoris minki*, *Agonoscena pistaciae*, mass rearing, release, pistachio.

**INTRODUCTION**

Pistachio production is of great economic importance in the Southeastern Anatolian Region, the main pistachio plantation area of Turkey. The common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Psyllidae) is a major pest of pistachio trees, *Pistacia vera* L. in Turkey since late 1980's (Mart *et al.*, 1995). Also, *A. pistaciae* is the most serious insect pest species of pistachio trees and causes considerable damage in Iran and Greece (Mehrnejad, 2001; Souliotes *et al.*, 2002), neighboring countries of Turkey. Overwintered psyllid adults appear on pistachio trees in mid-March and females lay eggs in mid-April and produce 5–6 generations a year (Çelik, 1981). Psyllid

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nymphs and adults interfere with kernel development, resulting in bud drop and defoliation. Both adults and immature suck plant sap and produce white crystallized honeydew. The control of this pest is difficult with insecticides because of its tendency to develop resistance to insecticides (Mehrnejad, 2001).

Anthocorids have been widely used in biological control for over a century and are considered as important natural enemies of pest species such as psyllids, thrips, mites and aphids (Lattin, 1999). *Anthocoris nemoralis* (Fabricius) (Hemiptera: Anthocoridae) has been released to control pear psylla in pear orchards (Fauvel *et al.*, 1994; Rieux *et al.*, 1994; Unruh and Higbee, 1994; Anonymous, 2015). *A. nemoralis* and parasitoid *Psyllaephagus pistaciae* Ferriere (Hymenoptera: Encyrtidae) were reported to be the most abundant natural enemies on pistachio trees in Makrakomi area of Central Greece (Souliotes *et al.*, 2002). The predator, *A. minki* is known as one of the native bio-control agents of *A. pistaciae* in pistachio production areas in Turkey (Bolu *et al.*, 1999; Çelik, 1981; Mart *et al.*, 1995; Yanik *et al.*, 2011a) and Iran (Pourali *et al.*, 2010). *A. minki* is highly active from early April to late October on pistachio trees (Bolu *et al.*, 1999).

The objective of this study was to evaluate effectiveness of *A. minki* as biological control agent of pistachio psylla. We believe this investigation is the first study on *A. minki* as a predator of the *A. pistaciae*. This study provides initial information for understanding the rearing and release of *A. minki*. Obtaining knowledge about this predator will help to reduce chemical applications against the pistachio psylla.

## MATERIAL AND METHODS

**Predator rearing.** *A. minki* adults were collected from pistachio orchards in Sanliurfa in 2005 and reared in the laboratory using predator *A. nemoralis*'s rearing method as described by Samsøe-Petersen *et al.* (1989) and Yanik and Uğur (2005), and kept under  $25\pm1^{\circ}\text{C}$ ,  $60\pm10\%$  RH and 16L8D photoperiod conditions. About 50-100 adults were placed in a plastic cup (500 ml). The lid of the cup contained a ventilation hole covered with a fine-mesh nylon screen. *E. kuehniella* eggs were supplied to the nymphs and adults as a nutrient source. Fresh bean pods (*Phaseolus vulgaris* L. (Fabaceae)) were added to each container as oviposition substrates. *E. kuehniella* eggs and fresh bean pods were replaced every two or three days. Fresh bean pods containing *A. minki* eggs were placed in a plastic container (250 ml) with a ventilation hole on its lid. *E. kuehniella* eggs were put near the fresh bean pods, food source for newly emerged nymphs. The diets and fresh bean pods were replaced every two or three days.

*E. kuehniella* was reared on a wheat and wheat-bran mixture at 2:1 ratio (Bulut and Kılınçer, 1987). The mixture was placed in plastic cups (27x37x7 cm) and *E. kuehniella* eggs were placed on the mixture for growth. Plastic cups were covered with a piece of gauze. After the emergence of *E. kuehniella* adults were transferred into a plastic cage (15x25x10 cm), having plastic screens on two

opposite sides, for mating and oviposition. Rearing of *E. kuehniella* were done at  $25\pm 1^{\circ}\text{C}$  and in constant darkness.

**Field releases.** A total of 5 500 *A. minki* adults were released into a 10 to 15 year old pistachio orchard of 220 trees in the area of Ogutcu, Sanliurfa (ca. 147 trees per ha, avg. height 2.55 m, tree canopy diameter 2.64 m, 6.4 extension shoot,  $n=10$ ) on different dates, May 4 and 24 (7 adults per tree), June 22 (4 adults per tree) and July 12 (7 adults per tree). Age and sex ratio of released adults 3-7 day-old and 1:1, respectively. A total of 25 *A. minki* adults were released per tree. First release was performed when approximately 30-40% of the eggs from overwintered female psyllas were hatch. In the laboratory, adults of *A. minki* were put in a small plastic cups, sawdust were provided for hiding. In the field each cup was emptied onto branches of tree. Ten trees were assigned for treatment and marked. Leaf and beating samples were taken at ten day intervals from May to November to assess the effect of the releases. Since trees were young, only a total of 20 leaves were picked from the marked trees and sides of a tree were reversed each week to minimize arthropod distribution. Leaf samples were taken to the laboratory in cooling boxes and immediately checked for arthropods under a stereomicroscope. A beating sample consisted of dislodged arthropods from tree branches were taken. After counting the beating samples, arthropods were released to the orchards.

No insecticide was applied in the release orchard. However, a fungicide Dodine was applied to trees for *Septoria pistaciae* disease on May 20 and June 4, 2006. An orchard with 200 trees next to the release orchard was selected as a control orchard (Control-I). No release was made, but only fungicide (Dodine) was used in Control-I orchard. Another orchard with 120 trees and 150 m away from the release orchard was used as the second control (Control-II). In addition to the same fungicide, trees received two insecticide (Phosalone) applications against pistachio psylla in Control-II orchard on June 8 and July 28, 2006. Due to the considerable defoliation and honeydew production, counting of psylla nymphs and eggs was stopped in early August in Control-II orchard.

**Determination of overwintering predator insects.** Tree bands of corrugated cardboard were used to provide shelters (Civolani and Pasqualini, 2002; Horton and Lewis, 2000; Horton, 2004) for overwintering *A. minki* and other predator insects. Each band was 7 cm wide and long enough to encircle the trunks of pistachio trees. Corrugations were 4 X 5 mm, large enough to allow insects fit in. Bands were placed around the trunks, 0.3 m above the ground. Nine trees were banded with corrugated cardboard traps in the release orchard on November 9, 2006. The traps were collected on February 22, 2007 and placed in large plastic bags. Bags and bands were checked every 3 or 5 days for predators in the laboratory from late February to early July. Insects were aspirated from the plastic bags and counted.

The correlation between *A. minki* and *A. pistaciae* was evaluated using linear regression model.

## RESULTS AND DISCUSSION

**Field releases.** The results indicated that there was a significant positive relationship between pistachio psylla and *A. minki* in the release orchard ( $F_{2,157}=20,2659$ ,  $P<0,01$ ) (Figure 1). Predator populations increased as the psylla population increased. Releases on May and June led the number of predator to increase by  $14.93\pm3.97$  adult + nymph/beating in the beginning of July and psylla population decreased from  $175.6\pm50.38$  to  $31.3\pm13.75$  nymph/composite leaves in the second week of July.

However, due to psylla infestation from an adjacent orchard (another side of release orchard, apart from Control-I orchard) o in the second week of July, psylla population increased up to  $193.4\pm39.67$  nymph/composite leaves in the release orchard in the first week of August. Therefore, predators were released for the fourth times in the release orchard on 12 July 2006. Psylla population was reduced to  $25.1\pm7.07$  nymph/composite leaves in the last week of August, after six weeks from the fourth release.

The highest number of psylla nymphs in Control-I was  $539.40\pm91.57$  nymph/composite leaves and occurred in the second week of October; while it was only  $70.4\pm20.67$  nymph/composite leaves in the release orchard.

Significant differences between release orchard and Control-II orchard in terms of psylla populations occurred one week after the second release. At the beginning of June, there was about  $370.60\pm32.28$  psylla nymphs/composite leaves in Control-II orchard.

Insecticide application reduced the number of psyllids to  $52.0\pm15.17$  nymph/ composite leaves in the second week of June. However, psyllid population increased at the end of July and reached  $458.00\pm56.53$  nymph/composite leaves and an additional insecticide application reduced the psyllid population to  $135.83\pm23.96$  nymph/composite leaves at the beginning of August.

Population of psylla did not decrease below economical threshold level in Control-II orchard. The highest number of *A. minki* was  $0.44\pm0.12$  adult + nymph/beating in Control-II orchard, which was considerably lower than those in the release orchard.

**Other insect natural enemies.** Total numbers and the occurrence rates of insect natural enemies of pistachio psyllid in the release and control orchards between May and November are shown in Table 1. *A. minki* was the most abundant species in the release orchard, followed by the nymph parasitoid *Psyllaephagus* sp. (Hym.: Encyrtidae) and Coccinellidae species. *Psyllaephagus* sp. was more abundant in the control orchards considering the other natural enemies. Compared to the release orchard, *A. minki* numbers were lower in Control-II orchard due to insecticide applications. Some of the natural enemies that were observed in the other orchards were not found in Control-II orchard.

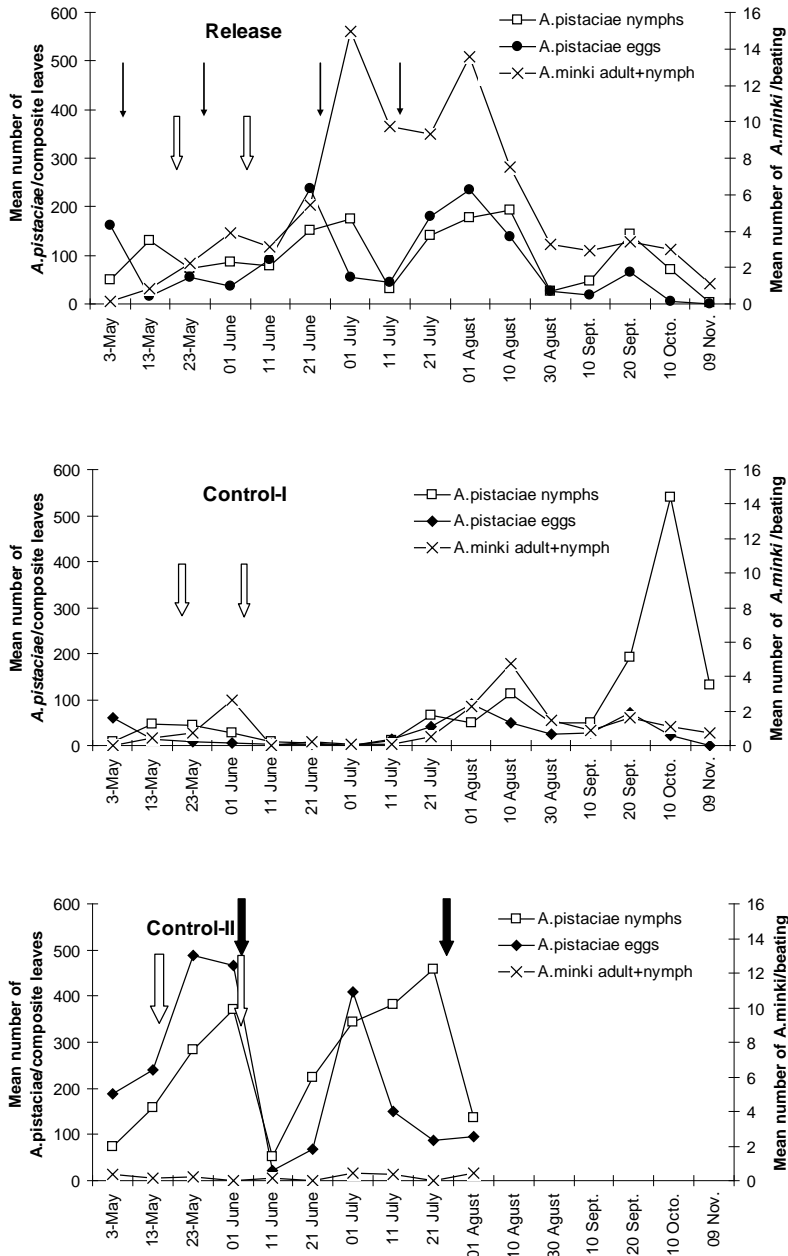


Figure 1. Mean number  $\pm$  SE of *Agonosцена pistaciae* nymphs, eggs/composite leaves and *Anthocoris minki* adult+nymph/beating, during the year 2006 ( $\downarrow$  arrow-release of *A. minki*,  $\downarrow$  open arrow-fungicide application,  $\downarrow$  bold arrow-insecticide application)

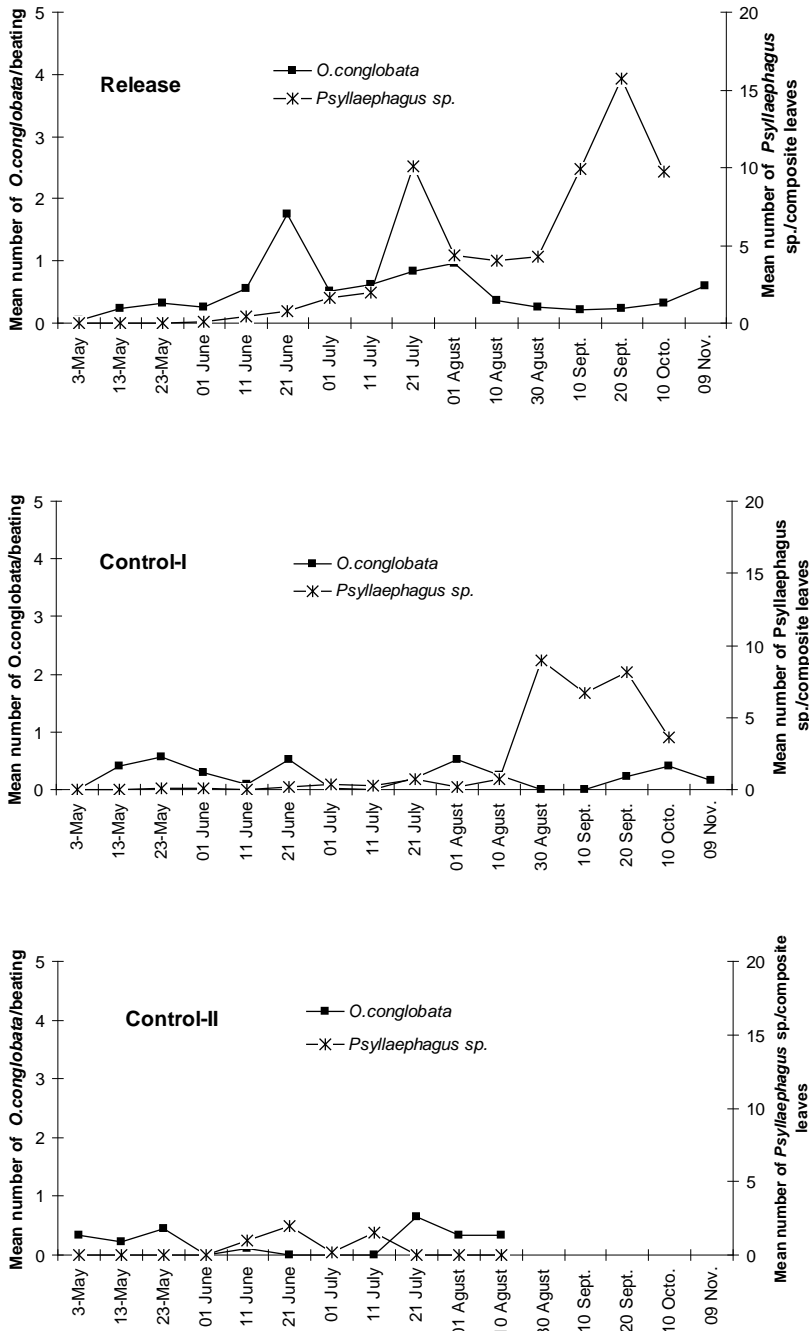


Figure 2. Mean number  $\pm$  SE of *Oenopia* (*Synharmonia*) *conglobata*/beating and parasitized nymphs by *Psyllaephagus* sp./composite leaves during the year 2006

Table 1. Total number and occurrence rates of insect natural enemies in release and control orchards between May and November

Natural enemies	Release orchard		Control-I		Control-II***	
	Total Number	%**	Total Number	%	Total Number	%
<i>Anthocoris minki</i> (Anthocoridae)	5 066	73.60	522	40.25	12	22.64
<i>Orius horvathi</i> (Anthocoridae)	49	0.71	15	1.15	0	0
<i>Piocoris luridus</i> (Lygaeidae)	12	0.17	9	0.63	0	0
<i>Campylomma lindbergi</i> (Miridae)	54	0.78	48	3.70	0	0
Coccinellidae*	493	7.16	113	8.71	13	24.53
<i>Chrysopa carnea</i> (Chrysopidae)	44	0.64	4	0.31	0	0
Parasitized nymphs by <i>Psyllaephagus</i> sp. (Encyrtidae)	1 258	18.27	586	45.18	28	52.83

\* *Oenopia* (*Synharmonia*) *conglobata* (most abundant species), *Hippodamia variegata* and *Coccinella septempunctata*

\*\* %=(Total number of insect x 100)/Total number of column

\*\*\* Insecticide used

Population changes of other relatively abundant predators (*Psyllaephagus* sp. and *Oenopia* (*Synharmonia*) *conglobata* (L.) (Coleoptera: Coccinellidae) in the orchards are shown in Figure 2. In the release orchard, population of parasitized nymphs by *Psyllaephagus* sp. increased when the population of *A. minki* decreased after mid July and in the beginning of September and decreased when population of *A. minki* increased in the beginning of August (Figure 1 and 2), indicating a negative relationship between the two natural enemy populations.

Total number of hatched and live eggs of *A. minki* per composite leaves is shown in Fig. 3. Number of live eggs reached the highest level ( $5.85 \pm 2.36$ ) in July, and decreased starting from August and no live eggs were observed after the middle of September.

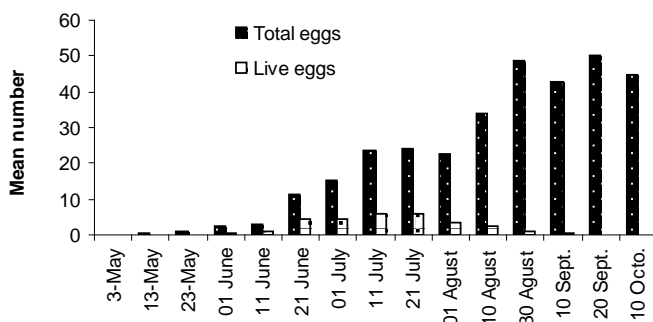


Figure 3. Mean number  $\pm$  SE of *Anthocoris minki* total and live eggs per composite leaves in release orchard

**Overwintering predators.** The numbers of *A. minki*, *O. conglobata*, *Piocoris luridus* (Fieber) (Heteroptera: Lygaeidae) and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) obtained from the corrugated cardboard traps were 25, 34, 17 and 1, respectively.

## DISCUSSION

The most important predator of pear psylla, *A. nemoralis*, is mass reared by specialist companies and released for biological control of pear psylla in pear orchards (Fauvel *et al.*, 1994; Rieux *et al.*, 1994; Sigsgaard *et al.*, 2006a; Sigsgaard *et al.*, 2006b; Unruh and Higbee, 1994; Anonymous, 2015). No study is available on biological control of pistachio psylla by *A. minki*. However, this predator species was successfully reared in the laboratory using the method to rear *A. nemoralis* (Samsøe-Petersen *et al.*, 1989; Yanik and Uğur, 2005).

*E. kuehniella* eggs, commonly used to rear anthocorid species (Arijs and De Clercq, 2001; Bueno *et al.*, 2006; Fauvel *et al.*, 1994; Murai *et al.*, 2001; Samsøe-Petersen *et al.*, 1989; Yanik and Unlu, 2011; Yanik and Unlu, 2015), were used to rear *A. minki*. Since anthocorid bugs lay their eggs in the tissue of plants, faba beans have been commonly used as the oviposition substrate for *Orius* spp., the anthocorid predators (Castañé and Zalom, 1994; Linus *et al.*, 2002; Murai *et al.*, 2001; Ruberson *et al.*, 1991; Richards and Schmidt, 1996; Venzon *et al.*, 2002). Faba beans that can be obtained throughout the year, an advantage for mass rearing (Murai *et al.*, 2001), were also successfully used as the oviposition substrate for *A. minki*.

Anderson (1962) reported that *A. minki* prefers psyllids as prey. Al-Marooof (1990) stated that poplar psylla (*Camarotoscena speciosa* Flor (Hemiptera: Psyllidae)) was an important pest of the poplars and *A. minki* was the most common predator species and constituted 81% of the predators. Besides, *A. minki* was an important and effective predator of Pemphigid aphids that cause galls on *Populus nigra* and *P. nigra* var. *italica* in Europe (Foster, 1990; Urban, 2002; Urban, 2004). Yayla (1983, 1984) reported that *A. nemoralis* was the most effective predator of the olive cottony aphid (*Euphyllura olivina* (Costa), Hemiptera, Aphalaridae)), while *A. minki* was the second important predator. Clearly, *A. minki* feeds on psyllids and aphids and the results of this study indicated that it is capable of suppressing the pistachio psylla.

Souliotes *et al.* (2002) indicated that *A. nemoralis*, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and endoparasite, *P. pistaciae* have an important role in biological control of *A. pistaciae* and there is a positive relationship between the population of *A. nemoralis* and *A. pistaciae*. The result of our study also revealed a positive relationship between *A. minki* and *A. pistaciae* in the pistachio orchards (Figure 1). The first release was performed on May 4 when 30-40% of the eggs from overwintering psyllids hatched. Second release increased the *A. minki* population above the naturally occurring level and kept the pest population at the economical threshold level in the second half of July (the economical threshold level; 25-30 nymph/composite leaf, Mart *et al.*,



(1995)). The populations of psylla nymphs were reduced by *A. minki* by 51% nine days after the second release and by 82% three weeks after the third release.

Although the Control I orchard was near the release orchard, psyllid population was rather low until September. According to long years of observations, psylla population ranges from orchard to orchard, and from tree to tree as well. Starting from September psylla population increased in Control I orchard. Early defoliation of pistachio trees caused by high psylla population brought about to drop fruit buds too, on which fruit of next year would develop.

Due to immigration of gravid adult psyllids from the unmanaged adjacent orchard, psylla population began to increase in release orchard after the second week of July. Because the release orchard was small, the immigration of adult psyllids from adjacent orchards onto the experimental plots was a risk. The fourth release decreased the pest population to 25.1 nymph/composite leaf level within six weeks, which is the lower limit of the economical threshold for this pest. Sisgaard *et al.* (2006a, 2006b) showed that during the release of *A. nemoralis* and *A. nemorum* (Linnaeus) (Hemiptera: Anthocoridae) in small experimental fields, immigration of *Cacopsylla pyri* Linnaeus (Hemiptera: Psyllidae) from an adjacent orchard reduced the effectiveness of *A. nemoralis*. Authors suggested that more than one release was necessary to maintain predators at higher population levels in release orchards.

Although, the number of psylla nymphs decreased immediately after the insecticide applications in Control II, it increased rapidly afterwards (Figure 1). Due to the pesticide applications, the number of natural enemy species and their density remained lower in the Control-II than in the release and the Control-I orchards (Table 1). Despite the two insecticide applications, *A. pistaciae* population did not decrease to the economical threshold level in Control-II orchard.

Due to the releases, the number of *A. minki* was the highest in release orchards, whereas the number of parasitized nymphs by *Psyllaephagus* sp. was the highest in control orchards (Table 1). This negative relationship between *A. minki* and *Psyllaephagus* sp. could be due to the fact that *A. minki* feeds on parasitized nymphs by *Psyllaephagus* sp.. Parasitoids appeared after the first week of June and reached the highest population density in late September and early October (Figure 2) when the ratio of parasitized and survived nymphs of *A. pistaciae* is approximately 1:10. This not significant contributes to the limitation of the population of *A. pistaciae*. According to Souliotis *et al.* (2002), the ratio of parasitized with *P. pistaciae* and survived nymphs of *A. pistaciae* is approximately 1:1 and this ratio contributes to the restriction of the population of *A. pistaciae* at the end of September/beginning of October.

*A. minki* females lay their eggs in the tissues of plants such as leaves throughout the season. The average number of survived eggs per leaf was  $5.85 \pm 2.36$  in July (Figure 3). There were no survived eggs on leaves after the mid-September. This suggests that *A. minki* population may not increase even *A. pistaciae* population increases after the mid-September. The mean number of

eggs laid end of the season was  $48.55 \pm 11.97$  eggs/composite leaves. Sisgaard *et al.* (2006b) reported that the theoretical egg number per tree was 11 *A. nemoralis* eggs which halved the *C. pyri* population compared to not release trees.

Overwintered predator species in an fruit orchard may be potential sources of biological control in orchards (Yanik *et al.*, 2011b).

Establishment of released natural enemies in release area is important for the sustainability of biological control. In this case, the ability of biological control agent to overwinter within the orchard is a critical factor. *A. minki* was able to overwinter in the corrugated cardboard traps in the orchards, indicating that this species could survive on trees in winters.

As a result, one year release of *A. minki* against *A. pistaciae* led to effective control of this pest. Other predators can also contribute to decrease psyllid population. In this study *O. conglobata* and *Psyllaephagus* sp. may be as important as anthocorids in controlling psyllid. Overall, our findings may provide important background for biological control studies in pistachio orchards in the future, because this was the first rearing and release study using *A. minki*.

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