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Determination of releasing dosage of *Aphytis Melinus* Debach (Hymenoptera: Aphelinidae) for the biological control of California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) in Turkey

ABSTRACT

California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) is a key pest of citrus in all growing regions of Turkey. Mass releases of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) are conducted in Turkey for the biological control of the red scale. We studied the timing and releasing regime of the parasitoid.

The study was conducted in two orchards in Adana and Erzin (Hatay) in 2013 and 2014. Each orchard was divided in four equal areas. In each date we released 25000 and 50000 parasitoids per ha in two modalities: (i) 4 releases from April to June, (ii) multiple releases from April to October every 15 days.

There were no statistical differences in the scale infestation level between the two releases regimes. The infestation rate of the red scale on fruits before the release of *A. melinus* was 8% in Adana and 10% in Erzin; after releasing the infestation rate was 6% in 2013 and 4% in 2014 in Adana and 5% in 2013 and 5% in 2014 in Erzin. The population level of the red scale on the citrus leaves was different before and after release in both orchards. However, no significant differences were observed in the population level of the scale on branches.

Key words: *Aphytis melinus*, *Aonidiella aurantii*, biological control, Turkey

INTRODUCTION

The California Red Scale (CRS), *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) is a key pest in all citrus growing regions of Turkey (Anonymous 2008, Uygun, 2001). CRS has 3 to 5 generations per year depending on climate or the geographic region (ANONYMOUS, 2008; UYGUN, 2001). CRS is a pest of difficult to control because of its biology. Although it can be found in different development stages, during the winter about 50% of the population consist of adult females. CRS is a viviparous species, i. e. eggs hatches inside the female body. After first generation overlapping of other generations occurs during the vegetation periods

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and crawlers can be found anytime. CRS females produce 100-150 crawlers during their life span. The scale can be found in all parts of tree, such as trunk, branches, leaves and fruits. While sucking the plant tissue their phytotoxic saliva secretion damage the parenchyma cells of tissue (WASHINGTON & WALKER, 1990). However the major damage is caused by fruit infestation cause loss of commercial value. Summer oil application as a recommended IPM strategy and both registered or unregistered insecticide applications are not able to control all CRS crawler stages during the season. For this reason farmers faced difficulties in controlling the pest. It is highly possible to loss trees within 1-2 years if no control strategy is applied against CRS (ANONYMOUS, 2008; UYGUN, 2001).

Aphytis spp. (Hymenoptera: Aphelinidae) are one of the most important parasitoid genus used for biological control of armoured scales (Hemiptera: Diaspididae). Among the *Aphytis* species *Aphytis melinus* DeBach is a well-known and effective beneficial species for biological control of *A. aurantii*, which is also part of the beneficial entomofauna in Turkey. *Aphytis melinus* is not only parasitoid of *A. aurantii* but also it can parasitize several species of Diaspididae family, which are agricultural pest of several fruit crops (GARCIA *et al.*, 2016; COPPING, 2009).

Aphytis melinus is a small bright yellow colour parasitoid with low dispersal capacity due to its short distance of fly ability (ZAPPALA *et al.*, 2012). For this reason mass rearing and releasing of this species is very important for effective biological control of *A. aurantii*. *A. melinus* is an ectoparasitoid, females lay their eggs underneath the scale on scale body, not inside the body of the scale insect. They prefer to parasite second instar nymph or virgin females. After egg hatching wasp larvae starts to feed on scale body at the end they cause dead of scale insect. *Aphytis melinus* complete a generation within three weeks. Adult parasitoids can inflict mortality on scale population by host feeding. Adult parasitoids complete their life span in 2 weeks in natural conditions. *A. melinus* female is able to lay approximately 100 eggs during their life time (LLEWELLYN, 2002; MALIPATIL *et al.*, 2000).

In the recent years biological control has become an important measure in plant protection in Turkey (Anonymous, 2016). Natural enemies are imported from abroad for biological control in greenhouses in Turkey. Currently in Turkey only two species namely, *Cryptolaemus montrozieri* (Coleoptera: Coccinellidae) (BiyoAvcı) and *Leptomastix dactylopii* (Hymenoptera: Encyrtidae) (BiyoArı), have been rearing for biological control of Citrus mealybug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae) in Turkey since 1970.

The objective of the present study was to determine the releasing time and dosage of *A. melinus* to improve the biological control of CRS in citrus orchards in Turkey. This study will give an opportunity to complete biological control studies in

citrus growing area, aiming to expand biological control practice in citrus horticulture in the country.

MATERIALS AND METHODS

The study was carried out in two Washington Navel orange orchards infested by CRS 3.8 ha, 22 years old orchard situated in Erzin (Hatay) and 2.8 ha, 50 years old orchard situated in Adana. Each orchard was divided in 4 plots corresponding to 4 different releasing strategies applied (tab. 1). The CRS infestation rate was determined in 2012 at harvesting time by sampling 100 fruits (25 fruits x 4 trees) each plot. In 2013 and 2014, CRS infestation level was estimated according to GÜMÜŞ & UYGUN (1992). Before the release of *A. melinus* by sampling 120 leaves (30 leaves x 4 trees) and 24 branches (6 branches x 4 trees) (about 20 cm long with 1 cm diameter) each plot. All samples were taken from each of the 4 trees in different cardinal directions within tree canopy at 50-150 cm height.

Tab. 1 - Releasing strategy, timing, number of release and dosage of *Aphytis melinus*.

Releasing time	Number of release	Dosage/ ha
15 th of April – end of July	7 release	25000 adults
	7 release	50000 adults
April - May	4 release	25000 adults
	4 release	50000 adults

The density of the different development stages of CRS and the parasitisation level by *A. melinus* determined in October by collection 10 fruits out of 4 trees infested by CRS in each plot. The infestation rate of CRS at harvest was determined by sampling 100 fruits (25 fruits x 4 trees) from each plot. The infestation level of CRS on leaves and branches was also estimated in February to see infestation by random sampling on the same 4 trees in the releasing section (GOMEZ & GOMEZ, 1984). The two citrus orchards where the study was carried out were maintained under integrated pest management strategies before and after the release of *A. elinus* and the applied plant protection interventions are reported in tab. 2.

Tab. 2 - Plant protection interventions applied in the two citrus where the study was carried out in Erzin and Adana. (Pest and diseases, active ingredients of pesticides, dosage and application time).

Pest and Diseases	Erzin		
	2012	2013	2014
Winter application	Summer oil 1,2 l/ 100 l water 25/02/2012	-	-
California Red Scale	Buprofezin 65 cc/ 100 l water 13/06/2012	Aphytis release April - May April - July	Aphytis release April - May April - July
Citrus mealybug	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 15/07/2012	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 05/07/2013	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 25/06/2014
Rust Mite	Spirodiclofen 20cc/100 l water 23/07/2012	Spirodiclofen 20cc/100 l water 11/07/2013	Spirodiclofen 20cc/100 l water 01/07/2014
Mediterranean Fruit Fly Ceratitis capitata	Econex CC feromone trap 1,5 trap/da 16/08/2012	Econex CC feromone trap 1,5 trap/da 20/08/2013	Econex CC feromone trap 1,5 trap/da 18/08/2014
Rust Mite		Fenbutatin oxide 60cc/ 100 l water 08/09/2013	Spirodiclofen 20cc/100 l water 01/10/2014
Pest and Diseases	Adana		
	2012	2013	2014
Winter application	Summer oil 1,2 l/ 100 l water 28/02/2012	-	-
California Red Scale	Buprofezin 65 cc/ 100 l water 23/05/2012	Aphytis release April - May April - July	Aphytis release April - May April - July
Citrus mealybug	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 17/06/2012	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 25/05/2013	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 12/05/2014
Rust Mite	Spirodiclofen 20cc/100 l water 09/07/2012	Fenbutatin oxide 60cc/ 100 l water 18/07/2013	Spirodiclofen 20cc/100 l water 29/06/2014
Citrus mealybug	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 15/08/2012	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 22/08/2013	Beneficial insects BiyoAvcı C. montrozieri 10 ad/ tree BiyoArı L. dactylopii 10 ad/ tree 10/08/2014
Mediterranean Fruit Fly Ceratitis capitata	Econex CC feromone trap 1,5 trap/da 16/08/2012	Econex CC feromone trap 1,5 trap/da 14/08/2013	Econex CC feromone trap 1,5 trap/da 21/08/2014
Rust mite	Fenbutatin oxide 60cc/ 100 l water 18/09/2012	Fenbutatin oxide 60cc/ 100 l water 02/09/2013	Fenbutatin oxide 60cc/ 100 l water 13/09/2014

RESULTS

In 2012 before the parasitoid release the infestation rate (%) of CRS determined in each orchard in each section by examination of 100 fruits (tab. 3). The efficacy of *A. melinus* determined on fruit, leaf and branch counting by comparing the data to the infestation rate of CRS in 2013 and 2014 (tab. 3).

Releasing studies started from April in each studying year. However long term releasing plan could not be completed because of ageing problem of butternut squash in mass rearing procedure. In both years we could release *A. melinus* until July. The releasing regimes in the selected orchards divided in 4 plots. The pre and post release sampling were taken from 4 trees in the middle of the section. The

Tab. 3 - Infestation rates (%) of CRS on fruits, leaves and branches in the two studied orchards before (2012 October – 2013 February) and after the release (2013 and 2014) of *Aphytis melinus*.

YEARS	Releasing Time	ADANA					
		California Red Scale Infestation Rate (%)					
		Fruit (N=100)		Leaf (N=120)		Branch (N=24)	
		Releasing dosage/ha		Releasing dosage/ha		Releasing dosage/ha	
	25000	50000	25000	50000	25000	50000	
2012 October and 2013 February	Pre-release	8	8	20	18.3	16.7	12.5
	Pre-release	6	10	20	22.5	20.8	16.7
2013	April-July	6	7	12.5	13.3	8.3	12.5
	April-May	5	6	11.7	10.8	12.5	12.5
2014	April-July	5	4	10	13.3	8.3	12.5
	April-May	4	3	10.8	10.8	8.3	12.5

YEARS	Releasing Time	ERZİN					
		California Red Scale Infestation Rate (%)					
		Fruit (N=100)		Leaf (N=120)		Branch (N=24)	
		Releasing dosage/ha		Releasing dosage/ha		Releasing dosage/ha	
	25000	50000	25000	50000	25000	50000	
2012 October and 2013 February	Pre-release	11	9	24.2	25.8	16.7	12.5
	Pre-release	10	10	23.3	24.2	12.5	8.3
2013	April-July	4	5	15	16.7	8.3	12.5
	April-May	6	5	14.2	17.5	4.2	12.5
2014	April-July	7	5	12.5	13	12.5	12.5
	April-May	4	4	10	15.8	4.2	8.3

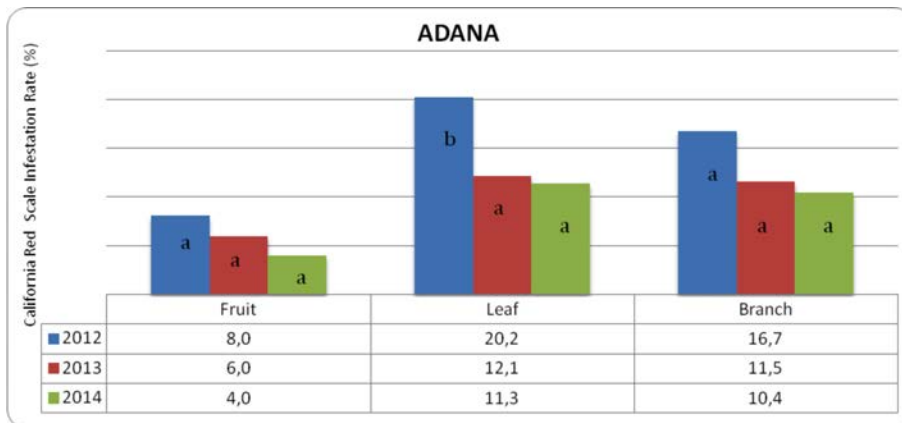


Fig. 1 - California Red Scale infestation rate on fruits, leaves and branches in Adana, before (2012) and after (2013 and 2014) the release of *Aphytis melinus*. (Means followed by the same letter do not differ significantly among the years based on LSD (0. 05) range test).

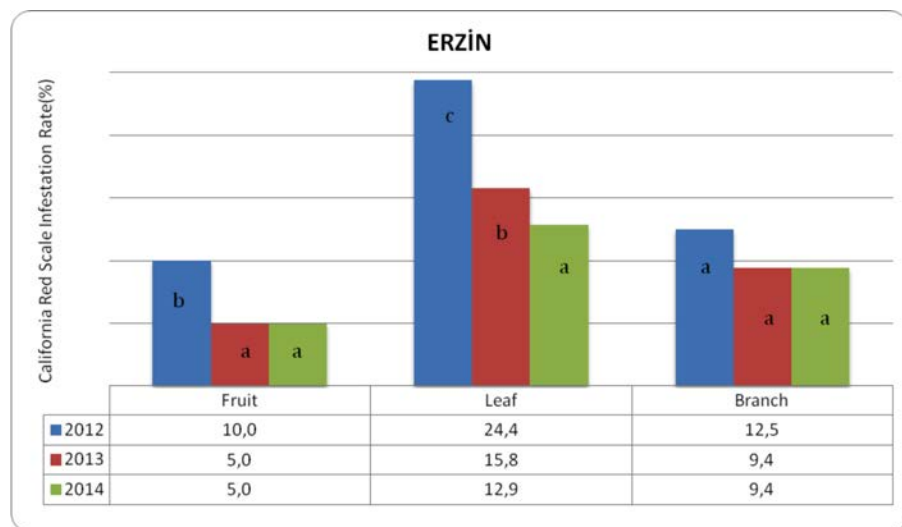


Fig. 2 - California Red Scale infestation rate on fruits, leaves and branches in Erzin, before (2012) and after (2013 and 2014) the release of *Aphytis melinus*. (Means followed by the same letter do not differ significantly among the years based on LSD (0. 05) range test).

evaluation of counting done by comparison of pre release year (2012) and post release (2013 and 2014) results. CRS infestation levels before (2012) and after the release of *A. melinus* (2013 and 2014) are presented in the tab. 3 and figs 1 and 2. There were no significant statistical differences between the two studied dosage rates (25000/ha and 50000/ha) according to LSD (0. 05) range test (tab. 3).

According to statistical analysis (LSD (0. 05) range test) between pre and post counting on fruit, leaf and branch, only statistical differences determined on the leaf samples in Adana and but in Erzin both fruit and leaf samples had been show differences (tab. 3 fig 1 and 2). However we could not find any statistical differences between releasing dosages. In the study conducted by MORENO & LUCK (1992) in different releasing dosages (annual mean release 4942 *A. melinus*/da; 9884 *A. melinus* /da and 19768 *A. melinus* /da) no differences had been found on fruit and branch counting. In both orchards the efficacy of *A. melinus* determined on fruits and leaves but not in branches is because of *A. melinus* prefer bigger individuals to lay their eggs, which usually occur on fruits and leaves (fig 1 and 2) (MORENO & LUCK, 1992). The CRS individuals on fruits and leaves are always bigger than the individuals on branches. Therefore the parasitisation rate is always higher on fruits and leaves (KARACA, 1998).

The analysis of our finding relies on CRS infestation rate on fruits but also the density of CRS development stages and parasitisation rate were examined on the 10 fruits. It seems that the parasitisation rate in October (2013 and 2014) was changed between 33% and 100% on 10 fruits (tab. 3). Due to CRS dispersal habits the infestation rate varied between 1 and 2 fruits out of 10 sampled fruits. CRS first stage crawlers after leave the female body in 6 hours time they have to settle in the most suitable place (EBELING, 1959). The crawlers of CRS tend to move to light direction and they move toward the leaves and fruits. Therefore the place of female determines the place of crawler. If there is a female near to fruit the crawlers tend to settle on fruit surface, or leaf or branch of tree. The result of this habit the individuals to settle on fruits and leaves are larger than the ones on branch or trunk, and also lead to higher parasitisation rates found on fruits and leaves rather than branch or trunk of tree (MORENO & LUCK, 1992).

CONCLUSION

The infestation rate of CRS should be the most important criteria in commercial citrus orchards likewise the *A. melinus* efficiency particularly on fruits and leaves seems to be important important. *A. autantii* infestation rate on the fruits was 8% in pre release counting in Adana, post release counting were 2% and 4% respectively in 2013 and 2014 (tab. 3 and fig 1). The efficacy rate of *A. melinus* was changed

between 75% to 50% in Adana orchard (tab. 4). In Erzin *A. aurantii* infestation rate on the fruits was 10% in pre release counting, post release counting were 4% and 5% respectively in 2013 and 2014 (tab. 3 and fig 2). The efficacy rate of *A. melinus* was changed between 40% to 50% in Erzin orchard (tab. 4).

The results suggest that the release of 25000 *A. melinus* release per ha in four launches between April and May was able to control CRS population.

Tab. 4 - In Adana and Erzin (2013 and 2014) *A. aurantii* development stages and parasitisation counted on 10 fruits.

Location	Year	Releasing Time	Releasing dosage (ha)	<i>Aonidiella aurantii</i> 10 fruit				
				Second stage larvae/ 10 fruit	Third stage larvae/ 10 fruit	Adult female/ 10 fruit	Parasitized Adult female/10 fruit	Parasitisation rate (%)
Adana	2013	April-July	25000	1	0.9	1.5	0.7	46.6
		April-July	50000	1.4	1.4	0.6	0.2	33.3
		April-May	25000	0.7	0.9	0.3	0.2	66.6
		April-May	50000	1.3	0.5	0.3	0.1	33.3
	2014	April-July	25000	1.6	0.3	0.2	0.2	100
		April-July	50000	0	0	0	0	0
		April-May	25000	1.7	0.4	0.3	0.1	33.3
		April-May	50000	1.1	0.8	0.6	0.3	50
Erzin	2013	April-July	25000	1	0.5	0.5	0.4	80
		April-July	50000	1.3	0.7	0.2	0.2	100
		April-May	25000	0	0.4	0.2	0.1	50
		April-May	50000	0	0.1	0.1	0.1	100
	2014	April-July	25000	0.6	0.2	0.3	0.2	66.6
		April-July	50000	0	0.2	0.1	0.2	100
		April-May	25000	1.2	0.3	0.3	0.1	33.3
		April-May	50000	0.9	0.6	0.2	0.1	50

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