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Impact of overwintering refugia of *Anagrus atomus* (Hym.: Mymaridae) on egg parasitism of grape leafhopper *Arboridia kermanshah* (Hem.: Cicadellidae)

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Abstract

Anagrus atomus (L.) (Hymenoptera: Mymaridae) is an egg parasitoid and an important mortality factor for the grape leafhopper, *Arboridia kermanshah* Dlabola (Hemiptera: Cicadellidae) populations in Isfahan vineyards. A study was conducted to characterize the plants in which *A. atomus* overwinters, dispersal of the parasitoid, and the level of parasitism of grape leafhopper eggs in two vineyards through 2000-2001. *A. atomus* overwinters in leafhopper eggs on rose, sweetbriar and blackberry. In a vineyard, with nearby overwintering refuges, the parasitoid emerged by mid-March from alternate hosts. It completed one generation on these alternate hosts, and then arrived in vineyard by late April, synchronized with grape leafhopper oviposition. The pattern of wasp colonization and parasitism indicated that parasitoids were more abundant in the vineyard with refuges close to it. In early spring, density of *A. atomus* was more than twice in this vineyard compared with vineyard with no refuges close to it. By mid season (July), *A. atomus* parasitism of *A. kermanshah* was significantly greater in the vineyard associated with refuges. Cumulative egg parasitism demonstrated that enhanced early season parasitism resulted in a better season-long increase in the mortality imposed by *A. atomus* on *A. kermanshah* eggs.

Key words: *Anagrus atomus*, *Arboridia kermanshah*, refugia, egg parasitoid, grape leafhopper, Isfahan

Introduction

The grape leafhopper (GLH), *Arboridia kermanshah* Dlabola, is an economically important pest of most vineyards of Iran (Mostaan & Akbarzadeh,

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1995; Latifian, 1998). This species overwinters as adult under leaves and in crop residues in Isfahan in center of Iran. It moves onto vines as soon as leaves appear and feeds on leaf sap of host plant. It has three generations per year and is most abundant from June to October. Few natural enemies have been reported for this pest (Latifian, 1998). An egg parasitoid of *A. kermanshah* in Isfahan was identified as *Anagrus atomus* (L.) (Hesami *et al*., 2001). This parasitoid completes development in 17 days at 26 ±2 °C and 40-60 % RH, a much shorter period than for development of its host. This suggests more generations of the parasitoid per generation of the host (Hesami, 2002). *A. atomus* is a cosmopolitan parasitoid of a variety of insect eggs (Huber, 1986; Triapitsyn, 1998) whilst *A. kermanshah* is distributed in West Palearctic region (Vidano & Arzone, 1987) and in Iran (Latifian, 1998), however this host- parasitoid relationshp did not study yet.

Anagrus species are valuable biological control agents of grape leafhopper species in Europe and North America (Arno *et al*., 1987; Cerutti *et al*., 1991; Corbett & Rosenheim, 1996; Williams & Martinson, 2000). The grape leafhopper species in Europe reported as *Empoasca vitis* Gothe, *Jacobiasca libyca* (Berg. and Zan.) and *Zygina rhamni* Ferrari that are different from Iran, but *A. atomus* is an important parasitoid in that region (Arno *et al.*, 1987; Cerutti *et al*., 1991). In Europe, high egg parasitization occurs in grapes near to wild vegetation where *A. atomus* overwinters and multiplies on alternate hosts (Cerutti *et al*., 1991). In Switzerland leafhopper species that lay eggs on roses and blackberries are important alternate hosts for this parasitoid (Cerutti *et al*., 1991) while *Erythroneura* species that are restricted to Nearctic region (Vidano & Arzone, 1987), are parasitized by *A. atomus* and other species of *Anagrus* (Doutt & Nakata, 1973; Williams, 1984; Williams & Martinson, 2000).

All grape leafhopper species overwinter as adults and all *Anagrus* parasitoids refuge for overwintering in host eggs (Cerutti *et al*., 1991; Doutt & Nakata, 1973; Williams, 1984; Williams & Martinson, 2000). Cerutti *et al*. (1991) reported that *A. atomus* appears to overwinter in leafhopper eggs mainly on roses and blackberries, and habitat management may be the most probable approach to enhance biological control of *E. vitis* populations. In North America, *Anagrus* spp. can overwinter in overwintering eggs of blackberry leafhopper, *Dikrella cruentata* Gill. (Doutt & Nakata, 1973; Williams, 1984); prune leafhopper, *Edwardsiana prunicola* (Edwards) (Corbett & Rosenheim, 1996; Murphy *et al*., 1996); white apple leafhopper, *Typhlocyba pomaria* McAtee (Seyedoleslami & Croft 1980) and also in diapausing insect eggs in *Acer saccharum* Marshall, *Robinia pseudo-acacia* L., *Rosa multiflora* Thunberg, *Salix nigra* L, *Vitis riparia* Michaux and *Zanthoxylum americanum* Miller (Williams & Martinson, 2000).

It is established that early season colonization by *Anagrus epos* Girault is greater in vineyards that are closer to riparian habitats (Doutt & Nakata, 1973; Williams, 1984). The effectiveness of French prune tree refuges in increasing control of the grape leafhopper had been evaluated (Corbett & Rosenheim, 1996; Murphy *et al*., 1996).

Morphology of immature stages and aspects of the biology of the *A. kermanshah* is reported only from Iran (Latifian, 1998) and morphology of *A. atomus* for different stages were reported by Hesami *et al.* (2001), however no information is available on interaction of *A. atomus* and *A. kermanshah*. Our study was aimed at evaluating the impact of *A. atomus* on *A. kermanshah* and determining the role of alternate hosts for *A. atomus* during winter and spring, and subsequent effects on parasitism of *A. kermanshah* in vineyards.

Materials and methods

Field sites

This study was conducted in a one hectare vineyard at the Isfahan University of Technology (IUT) (51°33' longitude and 32° 42' latitude) for two years of 2000-2001, and in another vineyard close to Steel Melting Factory, Zarin Shahr (SMF) (51° 23' longitude and 32° 26' latitude) 40 Km apart from IUT, for one year (2001). At the IUT site fruit trees (e.g. almond, apple, pear, apricot and mulberry) and ornamental plants (e.g. roses, sweetbriar and elms) were adjacent to vineyard. The IUT campus is also surrounded by shade trees and annual and herbaceous perennial wild vegetations. At the SMF site only pine, cypress, other vineyards and weeds were adjacent the vineyards. Samples were taken randomly. No insecticides were applied during the study in the vineyards.

Anagrus atomus overwintering sites

Initially leafhopper infested orchards were selected. In these orchards, shoots and branches of ornamental shrubs and fruit trees from rosaceous plants, close to experimental vineyards were collected from early December to early March 2000-2001. Ten different plant species were sampled. Overwintering leafhopper eggs are usually laid under epidermal tissue on younger branches and shoots of woody plants. Eggs are visible as a bean shape blister. Thirty centimeter apical length of shoots and branches comprised the sampling unit. On each sampling date, 20 samples were collected from each plant species. In the laboratory, shoots bearing symptom of leafhopper eggs (bean shape blister) were placed in wet cotton and held in a cage of paper carton. A glass vial was screwed at the top of the carton to allow light to enter and attract emerging parasitoids from leafhopper eggs. Cartons were held at 25 ±2 °C, 14:10 (L:D) photoperiod and 30-40% RH. The emerged adults were collected and indentified to species.

Monitoring of Anagrus atomus on roses in winter and spring

Many leafhopper (*Edwardsiana rosae* (L.)) eggs were found on roses. These eggs were collected during winter 2001 and dissected to determine if they were parasitized. Emergence of *A. atomus* on roses 100 meters away from the IUT vineyard was monitored twice weekly (late February- mid May 2001) with four yellow sticky traps (10 x 15 cm). Traps were coated with Tanglefoot adhesive; each attached to a 0.7 m long wooden pole and placed among roses and sweetbriars. In addition, 15 branches of 20-cm apical length were collected from roses twice weekly from mid February. These branches were surveyed in the laboratory for parasitoid emergence with counting the circular wasp exit holes.

Monitoring of Anagrus atomus and grape leafhopper adults in vineyards

To monitor adults of *A. atomus* and *A. kermanshah*, 5 yellow sticky traps were placed in each vineyard using 1.5 m wooden legs between planting rows. The traps was 10×15 cm and coated with Tanglefoot adhesive. The traps were replaced weekly from mid-April to late October. Number of *A. atomus* and *A. kermanshah* were recorded after examination of traps under a stereomicroscope.

Monitoring of grape leafhopper egg parasitism in vineyards

Parasitism of grape leafhopper eggs by *A. atomus* was assessed by weekly sampling of 3 leaves from 3 strata of each vine canopy as sample unit on ten vines, providing a total of 30 leaves per sampling date at each vineyard. Samples were taken from spring to autumn in IUT site for two years and for SMF site for one year.

In the laboratory, both sides of each leaf were examined for unparasitized and parasitized eggs (with a circular wasp exit hole or a clearly visible wasp larva or pupa within egg). The circular wasp exit hole becomes dry and brownish a few days after emergence. To measure parasitism, the previous exit holes were not counted.

Data analysis

Monthly data on grape leafhopper eggs, parasitoid adult and percentage parasitism of leafhopper eggs at the two sites were analyzed to compare differences of sites and effect of overwintering refugia. Data were transformed into log 10 to reduce variance heterogeneity. Variation between vineyards for numbers of parasitoid adults captured by traps, number of grape leafhopper eggs and parasitism percentage analyzed using a t-test by SAS software.

Results

Anagrus atomus overwintering sites

Rose, sweetbriar and blackberry were the only plants found with leafhopper eggs containing overwintering immature stages of *A. atomus*. The rose leafhopper, *Edwardsiana rosae* (L.) was the abundant species on these plants.

Cerutti *et al.* (1991) reared overwintered *A.atomus* from rose and blackberry in Switzerland. Williams and Martinson (2000) reared it from overwintering leafhopper eggs in *Rosa multiflora* Thunb. and *Ostrya virginiana* (Mill) in New York state. A number of studies indicate that Rosaceaeous plants play an important role in the overwintering of *Anagrus* species (Doutt & Nakata, 1973; Arno *et al*., 1987; Cerutti *et al*., 1991., Murphy *et al*., 1996, 1998; Williams & Martinson, 2000).

Monitoring of Anagrus atomus adults on roses in winter and spring

In 2001 in the IUT vineyard, the first adults of *A. atomus* were caught on yellow sticky traps in mid-March in roses. These numbers increased until early April and then declined. Another period of reduced flight activity occurred from late April to late May. Monitoring of parasitized eggs on rose showed a similar pattern (Fig.1). This indicated that *A. atomus* had at least two generations outside the vineyards, one from autumn to early spring (as diapausing larvae) and the other in spring. In Europe *A. atomus* emerges from overwintering host, completes one generation in leafhopper eggs primarily on blackberry and hazelnut in the spring, prior to dispersal into the vineyard (Cerutti *et al*., 1991). This is similar to other *Anagrus* species in North America (Doutt & Nakata, 1973; Williams, 1984; Williams & Martinson, 2000).

Monitoring of Anagrus atomus and grape leafhopper in vineyards

Abundance of *A. atomus* and grape leafhopper during spring-autumn in vineyards is shown in figures 2 and 3. The leafhopper adult population in the IUT vineyard was lower than the SMF site until mid-June. Populations simultaneously increased at both sites in late June and early July. The leafhopper peaked at 12 adults per trap in the IUT vineyard while it was more than 20 in the SMF vineyard. *A. atomus* adults were captured at the SMF site later than the IUT site. *A. atomus* peaked at 7 adults per trap in the IUT vineyard while it was less than 4 in the SMF vineyard. Although there is no significant difference between total numbers of *A. atomus* captured by traps throughout growing seasons between sites, wasp density in spring was higher at the IUT vineyard (Table 1).

Monitoring of grape leafhopper egg parasitism in vineyards

Data on leafhopper egg parasitism by *A. atomus* throughout the season is presented in figures 4 and 5 for the IUT and figure 6 for the SMF vineyards.

In the IUT vineyard, parasitized eggs were collected from mid–May, about two weeks after arrival of *A. atomus*, parasitism increased throughout June and July, and continued by late season. Except early May in which the initial oviposition by the overwintering leafhoppers was in progress, then after more eggs were parasitized and parasitism continued throughout the summer and early autumn (Fig. 4 and 5).

In the SMF vineyard, where a high number of the host eggs were laid from early May onward, parasitized eggs were collected in late May and parasitism increased throughout July (Fig. 6).

From late July, the impact of parasitoid was high at both locations, whereas the trend of parasitism in the SMF site was slower than the IUT site in late season (Table 1). Overall more leafhopper injury was observed in SMF site rather than IUT site.

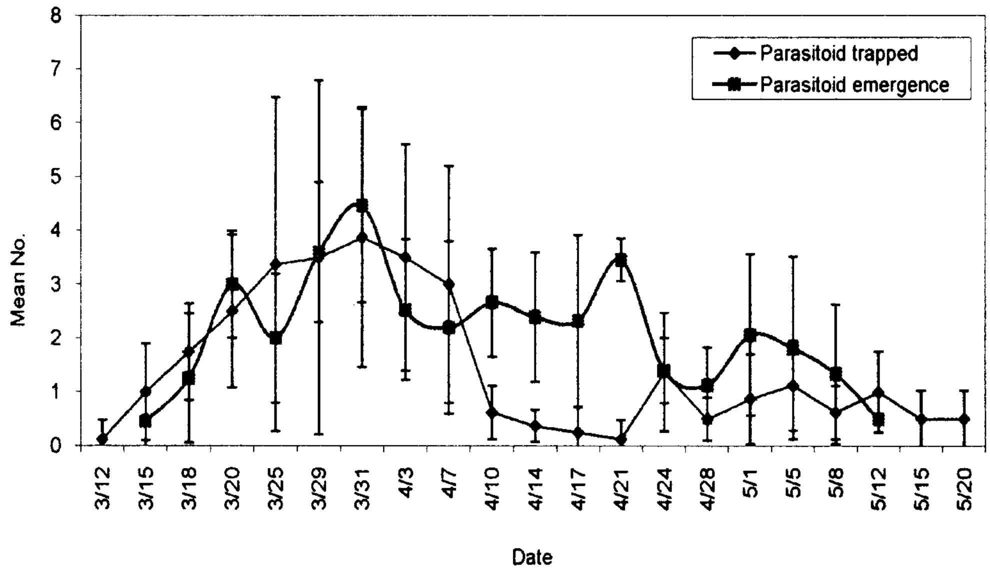

Fig.1. Population of *Anagrus atomus* captured by yellow sticky traps from roses and parasitoid emergence from rose leafhoppr eggs in IUT, 2001

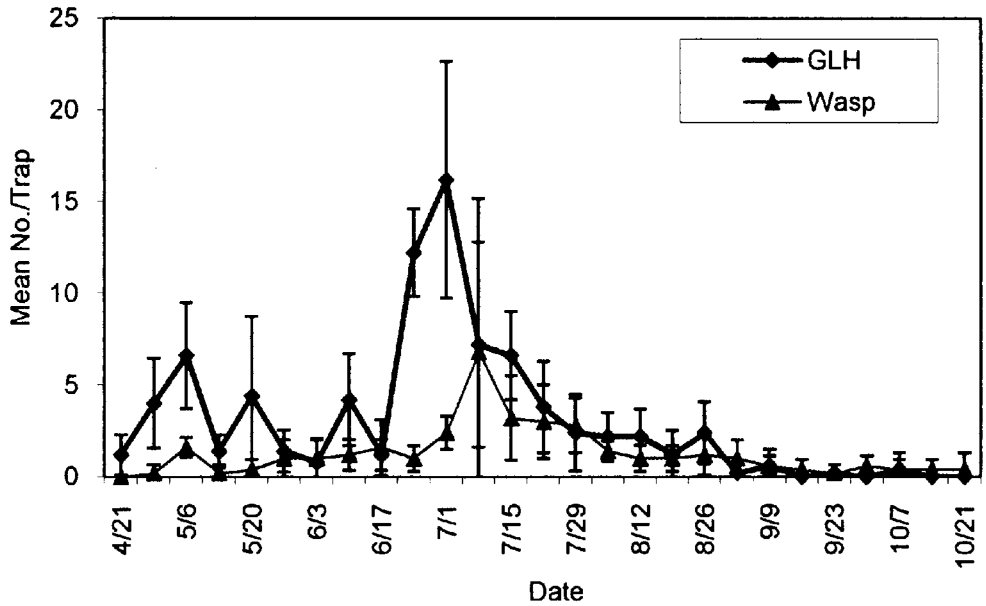

Fig.2. Seasonal trap catches of *Arboridia kermanshah* and *Anagrus atomus* adults in IUT, 2001

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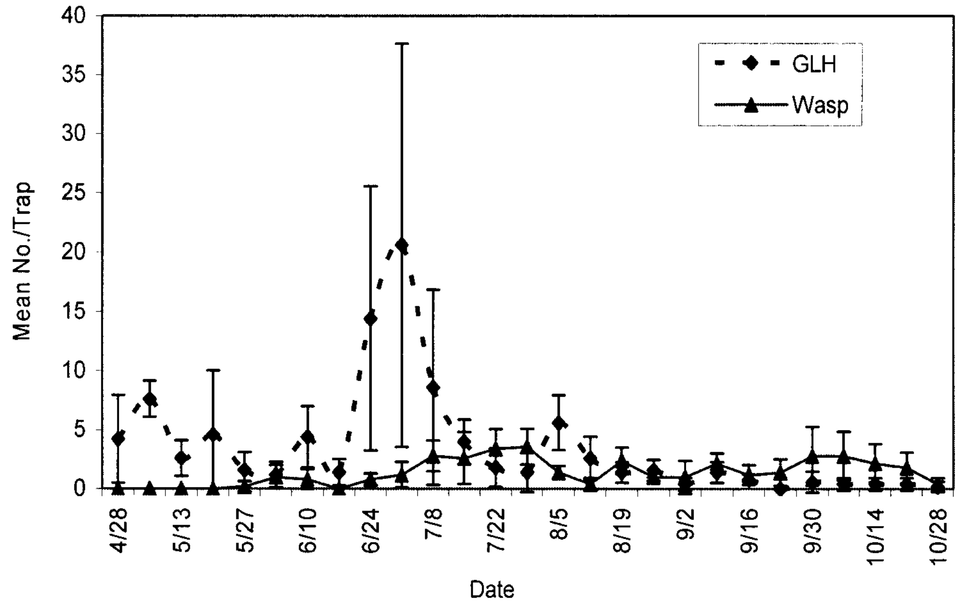

Fig. 3. Seasonal trap catches of *Arboridia kermanshah* and *Anagrus atomus* adults in SMF, 2001

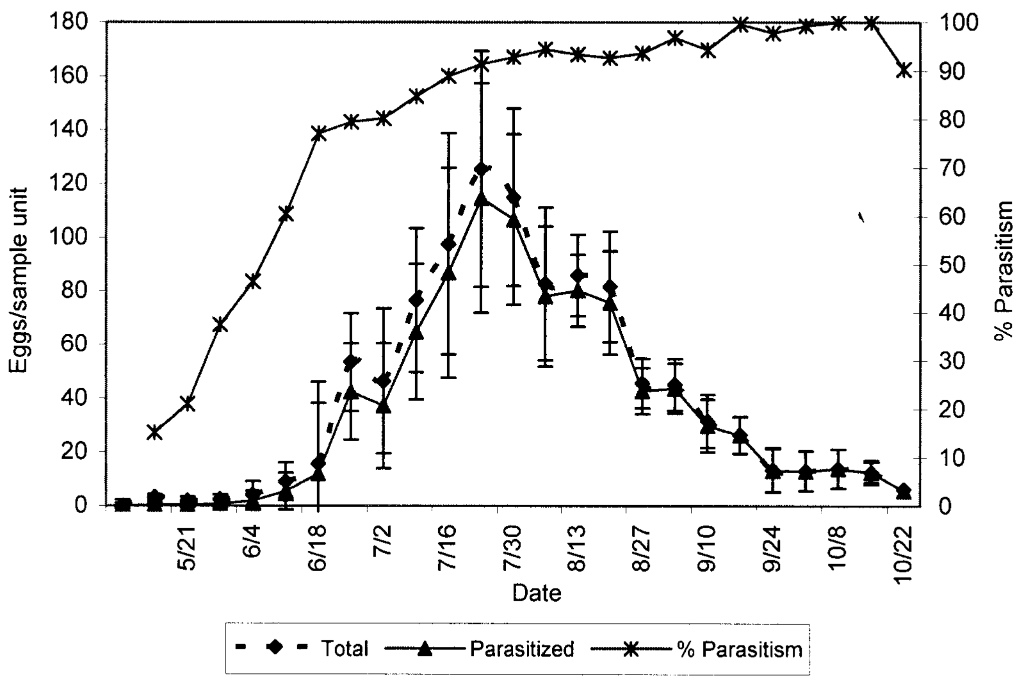

Fig. 4. Population of total and parasitized *Arboridia kermanshah* eggs in IUT,2000

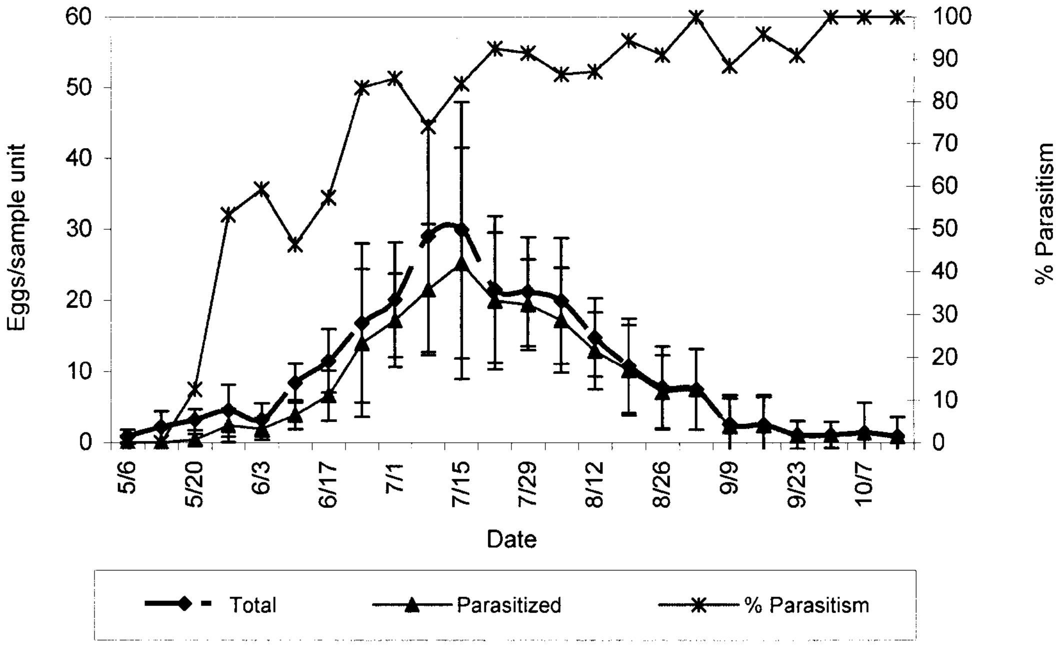

Fig.5. Population of total and parasitized *Arboridia kermanshah* eggs in IUT,2001

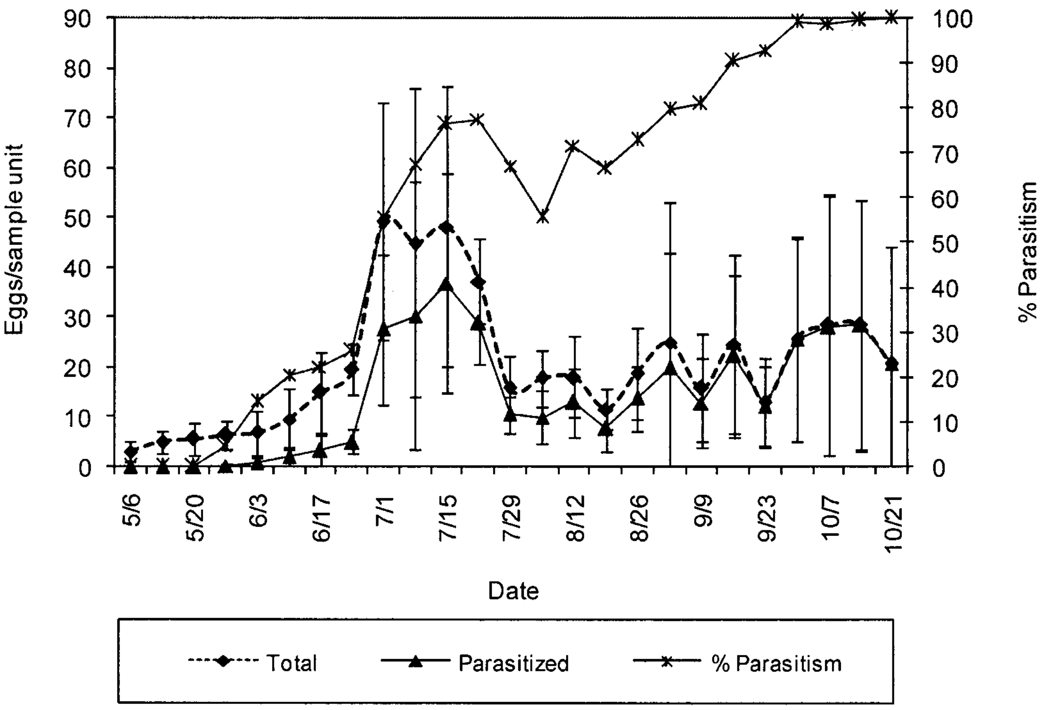

Fig.6. Population of total and parasitized *Arboridia kermanshah* eggs in SMF,2001

Table 1- Seasonal trap captures (Mean ± SE) of *Anagrus atomus* adults, grape leafhopper eggs per sample unit and percentage parasitism of grape leafhopper eggs and comparison by t-test between SMF and IUT vineyards (2001).

	Month	SMF	IUT	t-test
Total no. of parasitoid adults captured by traps	May	0.2 ± 0.4	2.6 ± 2.58	P< 0.05†
	June	3 ± 1.87	6.25 ± 0.83	P< 0.05
	July	14 ± 4.6	18.4 ± 8.4	ns‡
	August	6.73 ±3.58	5.75 ± 0.83	ns
	September	9.2 ± 4.62	2.8 ± 1.32	P< 0.01 §
	October	10 ± 3.93	1.25 ± 0.38	P< 0.01
	May-October	7.26 ±5.89	7.2±6.3	ns
No. of grape leafhopper eggs per sample unit	May	5.12±1.86	2.42±1.11	P<0.05
	June	12.82 ± 4.97	10 ± 4.98	ns
	July	38.96 ± 12.24	24.36 ± 3.92	ns
	August	16.85±3.08	13.35±4.54	ns
	September	21.3±5.68	2.92±2.28	P<0.01
	October	22.67±6.49	0.95±0.28	P<0.01
	May-October	20.42±12.69	9.39±9.09	P<0.01
Percentage parasitism of grape leafhopper eggs	May	1.15 ± 1.9	15.32 ±9.98	P<0.05
	June	20.9 ±4.1	61.85 ±13.48	P<0.01
	July	69.52 ±7.8	85.38 ±6.75	P<0.05
	August	68.27 ±7.25	89.35 ±3.03	P<0.01
	September	87.8 ±8.95	94.32 ±5.88	ns
	October	97.2 ±4.49	100	ns
	May-October	59.3 ±34.3	75.56 ±30	P<0.05

†: P< 0.05 = There is 95 % difference between two vineyards, ‡: ns= No significant differences between two vineyards, §: P<0.01= There is 99 % difference between two vineyards

Discussion

Our study has demonstrated that *A. atomus* can overwinter in the diapausing leafhopper eggs of *E. rosae* in rose, sweetbriar and blackberry. In Iran, Rose leafhopper is a common pest on rosaceous plants. Overwintering eggs are laid under epidermal tissue on younger branches and shoots of woody plants mostly on roses, sweetbriar, plum, and prune and rarely on other hosts. In spring adults disperse on pome and stone fruit trees and produce more generations (Davoodi, 1980). No overwintering leafhopper egg was found on other reported host plants in the orchards. Low density of leafhopper population and injury was usually observed in these orchards during growing season. Doutt and Nakata (1973), Williams (1984), Cerutti *et al*. (1991), Corbett and Rosenheim (1996), Murphy *et al*. (1996, 1998) and Williams and Martinson (2000) believe members of Rosaceaeous plants play an important role in the biology of *Anagrus* spp. It seems that in our study areas which blackberries are only rarely planted in orchards, roses and sweetbriars are important refuges. *Anagrus atomus* emerges from rosaceaeous alternate hosts in overwintering refuges in the spring and completes one generation prior to appearance of grape leaves. The role of alternate host is twofold in the spring. First, they preserve the parasitoid until the onset of grape leafhopper oviposition. Second, the availability of alternate hosts in the spring might allow *A. atomus* to increase before to disperse into vineyards and other habitats. Thus, leafhoppers that overwinter as eggs play an important role in the life history of the parasitoid, because they act as an ecological bridge, which spans the gap between times when leafhopper eggs are not available (Williams & Martinson, 2000). *A. atomus* parasitize other leafhopper species during growing season (Walker *et al*., 1997) and effect of these hosts can not be underestimated on overall population dynamics of this host-parasitoid life system.

Our results, showed that presence of alternate hosts, such as leafhoppers on the Roseacous plants in the IUT vineyard, were associated with more *A. atomus* capture in early season, compared with vineyard lacking overwintering refuges. Parasitoid abundance appeared to be more synchronized with the appearance of leafhopper host in vineyard with overwintering refuge nearby. Based on our visual observation, this probably causes to keep grape leafhopper injury below a specific threshold in the IUT vineyard compared with the SMF vineyard.

Corbett and Rosenheim (1996) hypothesized a windbreak effect generated by prune trees causes increased colonization by external *Anagrus* immediately downwind of refuges. Using elemental labeling techniques found that *A. epos* emerging from overwintering habitats external to the vineyard/French prune tress system were colonizing at a higher than average rate downwind of the wind break as a result of the turbulance generated by prune trees. However further investigations are necessary to show windbreak effect in our area. Williams and Martinson (2000) also showed the effect of distance from woodlots (as refuges) on early season parasitism of grape leafhopper eggs. We can anticipate such effect for responsibility of IUT site parasitism. It would be expected that most of *A. atomus* colonizers of IUT vineyard were from overwintering habitats adjacent (100 m) to the vineyard. While in SMF vineyard, the parasitoids were from external habitats with a long distance (5-10 km) from the vineyard. However, where three generations of the leafhopper is present in our area (Latifian 1998;

Latifian *et al*. 2004) and high population of leafhopper is present in July (Figs. 2, 3, 4, 5 and 6), later on populations of the grape leafhopper rapidly decrease. This is associated with high egg parasitism. This parasitoid has a much shorter life cycle in comparison host (Hesami, 2000) and it seems to be a very active parasitoid of the grape leafhopper. Our evaluation of the impact of overwintering refuge on the *A. atomus - A. kermanshah* system indicates that there is a potential for conserving and augmenting the numbers of *A. atomus* that plays an important role in decreasing the population of the grape leafhopper in this area. Habitat management (Cerutti *et al*., 1991; Murphy *et al*., 1996, 1998; Williams & Martinson, 2000) may be one promising approach to enhance biological control of *A. kermanshah* populations in our area.

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تأثیر وجود پناگاه زمستانی زنبور
Anagrus atomus (Hym.: Mymaridae) بر میزان پارازیتیسم تخم
زنجرک مو *Arboridia kermanshah* (Hem.: Cicadellidae)

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چکیده

زنبور *Anagrus atomus* (L.) (Hymenoptera: Mymaridae) پارازیتوئید تخم و از عوامل مهم مرگ و میر زنجرک مو *Arboridia kermanshah* Dlabola (Hemiptera: Cicadellidae) در تاکستانهای اصفهان است. در این مطالعه گیاهانی که زنبور روی آنها زمستانگذرانی می کند، پراکندگی پارازیتوئید و میزان پارازیتیسم تخم های زنجرک مو در دو تاکستان طی سالهای ۱۳۷۹ و ۱۳۸۰ بررسی گردید. زنبور *A. atomus* زمستان را درون تخمهای زنجرک گل سرخ روی رز، نسترن و تمشک به سر می برد. در یک تاکستان که در مجاورت خود میزبانهای زمستانگذران داشت، پارازیتوئید از دهه سوم اسفند از میزبان زمستانگذران خود خارج و یک نسل را در بهار روی آنها تکمیل کرد. از ابتدای اردیبهشت و همزمان با تخمگذاری زنجرک مو، این زنبورها وارد تاکستان شدند. الگوی تشکیل کلنی و پارازیتیسم زنبور نشان داد که پارازیتوئید در تاکستانی که در مجاورت خود میزبان زمستانگذران داشت فراوان تر بود و در اوایل فصل تراکم زنبور پارازیتوئید بیش از دو برابر تاکستانی بود که میزبان زمستانگذران در اطراف خود نداشت. در اواسط فصل میزان پارازیتیسم تخم زنجرک مو در تاکستان با محلهای زمستانگذران به طور قابل توجهی بیشتر بود. پارازیتیسم تجمعی مشخص کرد که افزایش پارازیتیسم در اوایل فصل منجر به افزایش تلفات تخم های زنجرک مو توسط زنبور پارازیتوئید تخم در طول فصل می گردد.

واژه های کلیدی: *Anagrus atomus* ، *Arboridia kermanshah* ، پناهگاه، پارازیتوئید تخم، زنجرک مو، اصفهان.