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Seasonal dynamics of: the pea aphid, *Acyrthosiphon pisum* (Harris), its natural enemies the seven spotted lady beetle *Coccinella septempunctata* Linnaeus and variegated lady beetle *Hippodamia variegata* Goeze, and their parasitoid *Dinocampus coccinellae* (Schrank)

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Abstract: Two important lady beetle species commonly found in alfalfa fields in Iran are the variegated lady beetle *Hippodamia variegata* Goeze and the seven spotted lady beetle *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae). These two species attack many aphid species including the pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae). In this study, the seasonal population changes of *A. pisum*, *H. variegata*, *C. septempunctata* and the parasitoid, *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) were studied in alfalfa fields in the 2012 and 2013 seasonal periods. The highest ladybird densities were noted on July 5, 2012 and on September 6, 2013 (17.2±2.8 and 13.4±1.6) individuals per 20 sweeps, respectively. Parasitism rates by *D. coccinellae* ranged from approximately 3 to 6% in two subsequent years, respectively. Parasitism was higher early in the growing season. Most parasitised ladybirds were females. There was no significant relationship between the temperature and relative humidity with pea aphid populations, although the aphid populations declined during the hot summer period. In contrast, the relationship between temperature and the *H. variegata* population was significant and positive in both years of the study. It has been shown that these lady beetle species have a major role in reducing the pea aphid populations in alfalfa fields. Due to the relatively low percentage of field parasitism by *D. coccinellae*, this parasite might not reduce the biocontrol efficiency of lady beetle species.

Key words: *Dinocampus coccinellae*, lady beetles, pea aphids, population fluctuations

Introduction

Pea aphids, *Acyrthosiphon pisum* (Harris) are an important aphid species which mainly attack alfalfa fields and which feed on other legumes such as pea, broad bean, faba beans, and lentils. Pea aphids have been reported from different regions of the world (Grimm 1972; Summers 1976) including many parts of Iran (Monajemi and Esmaili 1981). Besides direct losses, *A. pisum* is an efficient vector and non-persistently transmits more than 30 viruses, including Pea Enation Mosaic Virus (PEMV) and Bean Leaf Roll Virus (BLRV) (Harris and Maramorosch 1977; van Emden and Harrington 2007). Lady beetles are among the most common natural enemies that attack this aphid species. Lady beetles maintain pea aphid populations well below the economic injury level (Capinera 2001; Isikber and Copland 2002; Obrycki *et al*. 2009).

The variegated lady beetle, *Hippodamia variegata* Goeze, is an efficient aphidophagous lady beetle in a diversity of crops. It feeds on at least 12 different aphid species including the cotton aphid, mustard aphid, black bean aphid, and the pea aphid (Obrycki and Orr 1990; El-Hag and Zaitoon 1996; Obrycki 1998; Farhadi *et al*. 2010; Madadi *et al*. 2011). The variegated lady beetle originated from the Palaearctic region, and the distribution was cosmopolitan (Krafsur *et al*. 1996; Franzman 2002), but now the distribution is widespread through the world (Franzman 2002) including all of Iran (Lotfalizadeh 2001).

The seven spotted lady beetle, *Coccinella septempunctata* L., is another common Palearctic lady beetle. This species has a broad geographical distribution and is widely recorded from Asia to North America (Elliott *et al*. 1996; Alyokhin and Sewell 2004). It is also reported from other world regions (Kawauchi 1983).

Many ecologists have studied the population dynamics of insect pests and natural enemies to find the most important agents and assess population suppression characteristics. Therefore, a study on the population dynamics of the pea aphid and its predators may clarify the role of lady beetles in reducing the pea aphid populations and also shed light on the potential of lady beetles to suppress pea aphid populations. Both lady beetle species are considered good candidates for biocontrol of alfalfa aphids. Together with parasitoids, both beetle species also mitigate outbreaks of the pea aphid.

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One factor that may decrease lady beetle efficiency in many regions is the occurrence of natural enemies, which attack beneficial predators. *Dinocampus* (syn. *Perilitus*) *coccinellae* Schrank is the most important solitary parasitoid of Coccinellidae reported from many regions of the world (Firlej *et al*. 2005; Berkvens *et al*. 2010; Minaar *et al*. 2014; Tavoosi Ajvad *et al*. 2014). It is a generalist endoparasitoid of Nearctic and Palaearctic Coccinellids (Ceryngier *et al*. 2012). Despite this importance, many studies do not provide information about *D. coccinellae* population fluctuations in relation to environmental factors (Riddick *et al*. 2009).

Population dynamics of alfalfa aphids and their natural enemies have been studied by many researchers in Iran as well as in other countries (Grimm 1972; Wheeler 1974; Neuenschwander *et al*. 1975; Summer 1976; Harper 1978; Gonzalez *et al*. 1979; Aeschlimann 1981; Monajemi and Esmaili 1981; Takahashi and Natio 1984; Rasoulian 1985; Nakashima and Akashi 2005; Rakhshani *et al*. 2006).

The first aim of this study was to increase the knowledge about the seasonal fluctuations of pea aphids and the two most common aphidophagous lady beetles, *H. variegata* and *C. septempunctata*. The second aim is to increase the knowledge about relationships of pea aphid and two lady beetle species to environmental factors in alfalfa crops (*Medicago sativa* L.). Furthermore, the dynamics and the negative effect of the parasitoid *D. coccinellae*, as a natural enemy of the two lady beetles (ignored in previous Iranian studies) were investigated.

Materials and Methods

Sampling

This research was carried out in an alfalfa research field (ca. 4.5 ha) at Bu-Ali Sina University, Hamedan (N35°1′, E48°31′) to determine the seasonal fluctuation pattern and population density of *A. pisum*, *H. variegata*, *C. septempunctata* and *D. coccinellae*.

Lady beetle adults and *A. pisum* were sampled weekly from May to October 2012–2013 (a total of 42 times), starting from the first emergence of the pea aphids until the third (last) alfalfa cut. The sampling unit was selected as 20 standard sweeps by an entomological net over the crop. At each sampling date, 20 samples were regularly taken (from 9 a.m. to 12 a.m.) across the field diameters. Three meters of field edge were not sampled to prevent an edge effect.

The collected lady beetles were brought to the laboratory and kept in a growth chamber under climate controlled conditions [21±2°C, 70±10% and 12 : 12 (L : D) h photoperiod] to estimate the parasitism rate by *D. coccinellae*. They were fed pea aphids, pollen and a 10% honey-water solution daily. The presence of parasitoid cocoons was recorded every two days and recording was stopped after 20 days since 20 days are sufficient for the development of *D. coccinellae* (Wright and Laing 1978; Obrycki 1989).

Statistical analysis

Correlation tests were used to find the pea aphid and lady beetle population relationship with the *D. coccinellae* population, and the meteorological factors (mean daily temperature, relative humidity, and weekly rainfall) as independent variables.

Results

Population fluctuation of *Acyrthosiphon pisum*

In the first year of the study, the first sampling was conducted on May 17, 2012; it is probable that the parthenogenic pea aphid females emerged earlier than this date. It was observed that the population mainly decreased and remained at a low level during August 2012. Afterward, the pea aphid population showed an increase and peaked at the end of September followed by a steep decline during October 2012 (Fig. 1).

The next year, the population fluctuations were completely different. The sampling of female aphids was started one month earlier and the pea aphid population

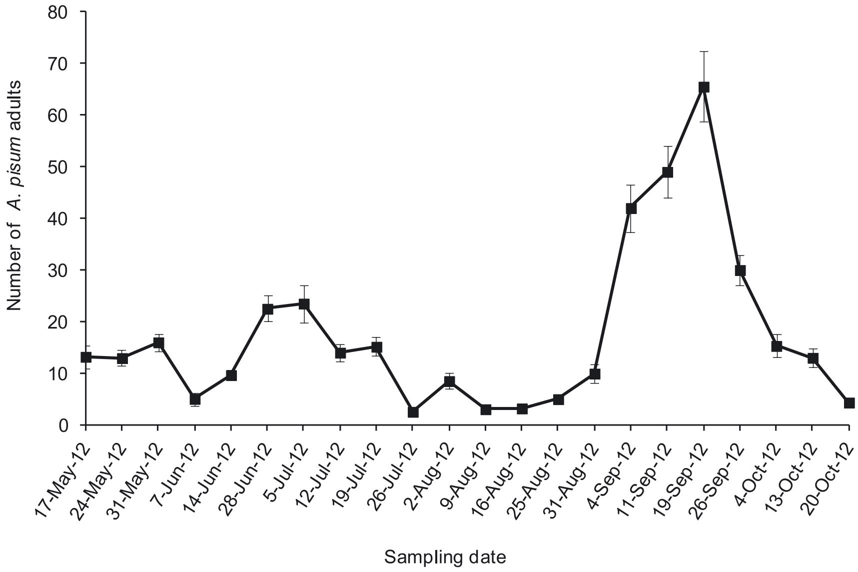

Fig. 1. Population fluctuations of *Acyrthosiphon pisum* adults in the first year of the study (the means±SE of 20 samples)

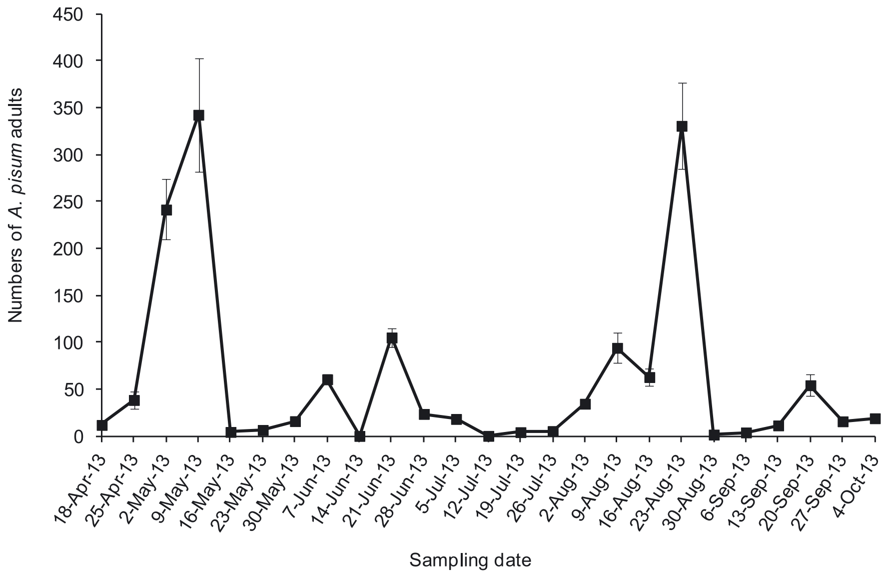

Fig. 2. Population fluctuations of *Acyrthosiphon pisum* adults during the second year of the study (the means±SE of 20 samples)

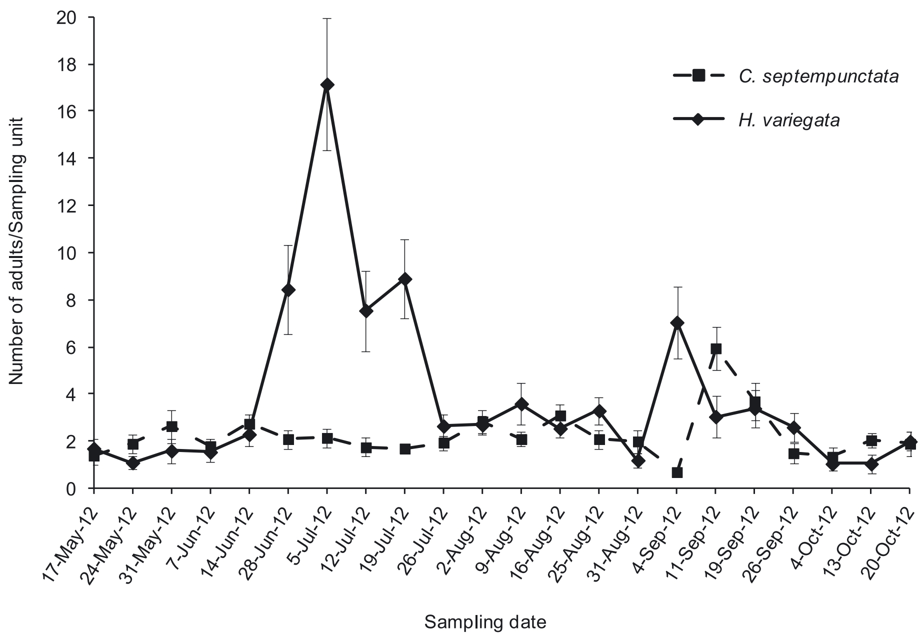

Fig. 3. The population trends of *Hippodamia variegata* and *Coccinella septempunctata* in the first year of the study (the means±SE of 20 samples)

showed high amplitude oscillations, with two peaks: May 9 and August 23, 2013 (Fig. 2). The mean respective densities of the pea aphid were 342.45±60 and 330.65±45.5/sampling unit adults. In both years, there was a drastic reduction in the population density of *A. pisum* from July to the end of August.

Population fluctuation of *Hippodamia variegata* and *Cocccinella septempunctata*

The first *H. variegata* adults were found on May 17, 2012 and reached a maximum on July 5, 2012 (mean relative abundance of 17.2±3.0 individuals/sampling unit). This species had another peak on September 04, 2012 which was much lower than the first one (7.1±1.5 individuals/sampling unit) (Fig. 3). The *Coccinellae septempunctata* population varied slightly. Only late in the season did *C. septempunctata* show a short peak (5.6±0.9 individuals per sampling unit).

In the second year, the *H. variegata* population showed three population peaks on July 5, 2013, September 6, 2013 and September 20, 2013 with a mean relative abundance of 11.9±1.8, 13.4±1.6 and 12.1±2.8 individuals/sampling unit respectively (Fig. 4). In the second year, there was an increase in the general level of *C. septempunctata* population and two noticeable peaks in population levels occurred on May 8, 2013 (8.2±2.0 individuals/sampling unit) and on September 6, 2013 (7.7± 0.8/sampling unit) (Fig. 4).

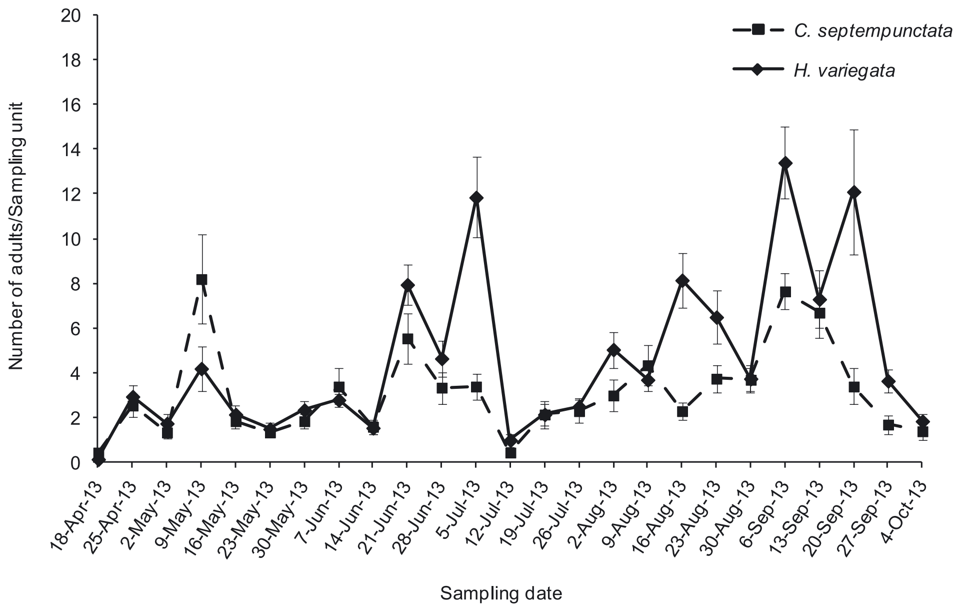

Fig. 4. The population trends of *Hippodamia variegata* and *Coccinella septempunctata* in the second year of the study (the means±SE of 20 samples)

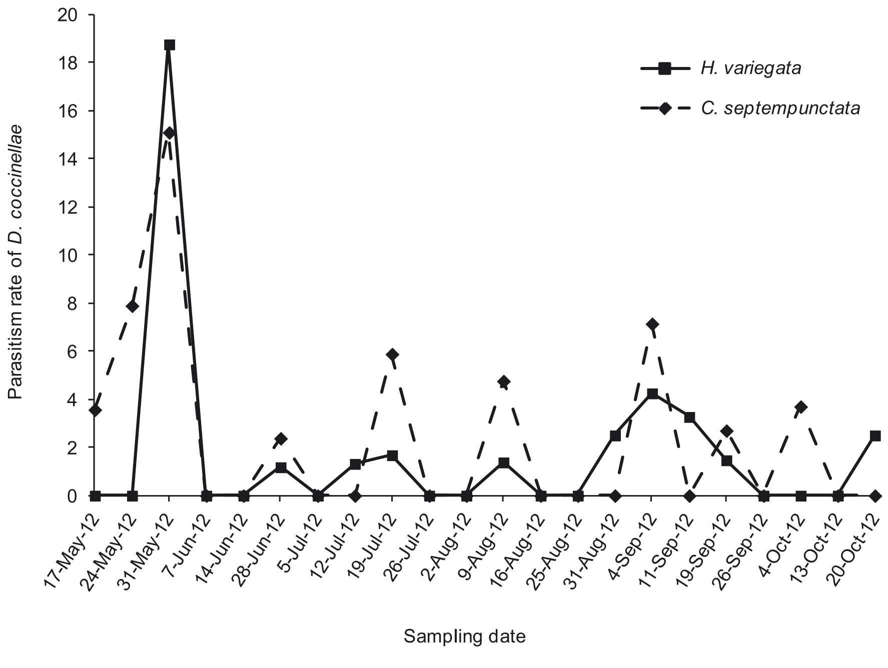

Fig. 5. The parasitism rate of *Dinocampus coccinellae* on *Coccinella septempunctata* and *Hippodamia variegata* in the first year of the study

In both years of the study, the *C. septempunctata* showed an overall lower population density than *H. variegata*. On the graphs, it can be seen, at least in the second season, that the population density of the two species varied synchronously, and peaks and depressions often occurred in the same samples. There were common environmental density drivers for both lady beetle species.

Dinocampus coccinellae population

The mean parasitism rate of *C. septempunctata* by *D. coccinellae* was estimated to be 5.9% in the first year of study. Of those individuals that were parasitised, 96% were adult females. The mean parasitism percent of *C. septempunctata* in the following year declined to 4.6%, and of those, 84% were adult females.

The mean parasitism rate of *H. variegata* by *D. coccinellae* was 4.1% and 5.8% in 2012 and 2013, and of those, 74 and 81% were adult females, respectively. The highest parasitism rate in the first year of the study was recorded on May 31, 2012 on both lady beetle species and the seasonal changes were synchronized (Fig. 5), while in 2013 the peaks for *C. septempunctata* and *H. variegata* were on May 2 and April 18, respectively and the changes were generally less synchronized (Fig. 6).

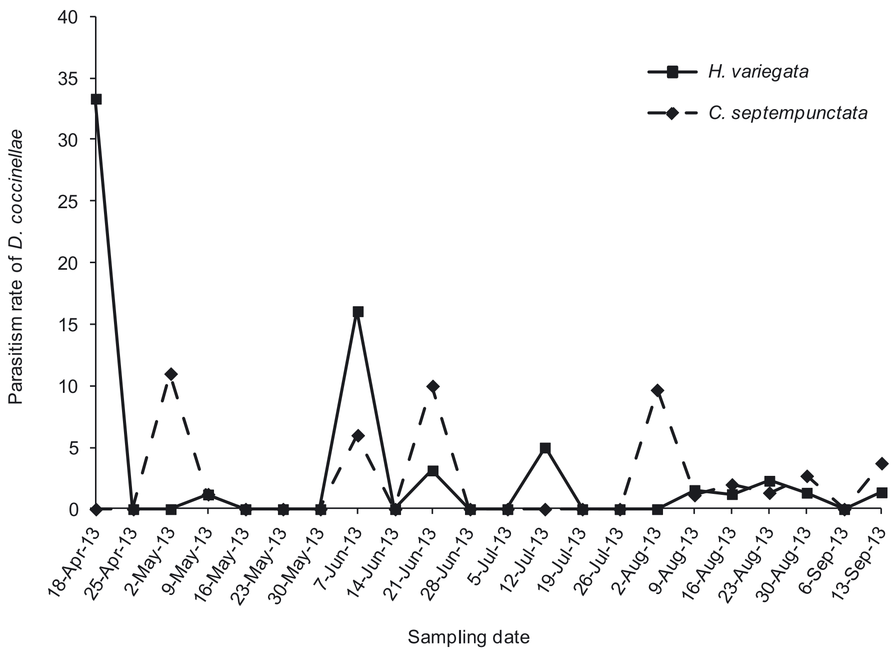

Fig. 6. The parasitism rate of *Dinocampus coccinellae* on *Coccinella septempunctata* and *Hippodamia variegata* in the second year of the study

Environmental factors and their relationships with *Acyrthosiphon pisum* and lady beetle populations

In both years of the study, we did not find any significant correlation between the daily temperature and the population density of pea aphid and *C. septempunctata*, on different sampling dates (Table 1). However, this correlation was significant and positive for *H. variegata* in both years. The correlation of humidity with the population density of all the species studied was insignificant, and in most cases this relationship was negative. It should be noted that the daily temperatures and relative humidity records were obtained from a station 30 km from the study site, and that may have influenced the correlation.

Correlation between aphid and lady beetle populations

There was a significant positive relationship between the population density of *C. septempunctata* and *A. pisum* in 2012 ($r = 0.41$, p-value = 0.049) and 2013 ($r = 0.38$, p-value = = 0.03), however this was not significant for *H. variegata* ($r = 0.21$, p-value = 0.33 and $r = 0.09$, p-value = 0.66) for 2012 and 2013, respectively.

Discussion

During this two-year study, the seasonal fluctuations of the pea aphid and the associated lady beetles, were examined. In both years and during the hottest months of the year, aphid population quickly declined. In the second year, the *A. pisum* showed typical aphid population dynamics that demonstrated an early increase in population size in spring typically followed by a steep decline in abundance during summer and again an increase in autumn. Dixon (1998) demonstrated that at high temperatures, the relative growth rate of aphids decreases while their mortality increases. The reduction in host plant quality during summer may also be responsible for the reducing trend of the pea aphid population. Similarly, in the present study, aphid natural enemy densities were reduced during the hot summer, while decreasing temperatures and an increase relative humidity at the end of summer resulted in a peak in the pea aphid population in both years. In the second year, the pea aphid population experienced sudden drops at the next sampling date. This drop may be attributed to the first and second crop harvesting. The harvesting significantly reduced both pea aphid abundance and the abundance of two lady beetle species (Soleimani, unpublished data).

Table 1. The relationship of environmental factors and the pea aphid, *Acyrthosiphon pisum* and lady beetle populations

Environmental factors	2012			2013		
	A.p.	H.v.	C.s.	A.p.	H.v.	C.s.
Temperature	–0.07(0.37)	0.43(0.02)	0.14(0.25)	–0.21(0.15)	0.39(0.02)	0.11(0.29)
Humidity	–0.07(0.36)	–0.23(0.14)	–0.11(0.31)	0.31(0.06)	–0.28(0.085)	0.001(0.49)

Correlation coefficients (p-value of) temperature and humidity with population of *Acyrthosiphon pisum* (A.p.), *Hippodamia variegata* (H.v.), and *Coccinella septempunctata* (C.s.)

In the current study, the levels of predator activity were very low at the beginning of May. As the aphid populations built up, though, the lady beetles became abundant and their population dynamics followed aphid population trends in a density dependent manner, especially during 2013. It seems that a reduction in aphid populations along with the higher temperatures during this period had a negative effect on the densities of natural enemies as well. This issue has also been addressed by Nakashima and Akashi (2005).

It has been shown that there is a significant correlation between the seven spotted lady beetle *C. septempunctata* and pea aphid populations. This significance may be assigned to the more important role of *C. septempunctata* in reducing the pea aphid in alfalfa fields.

Different population peaks of *A. pisum* have also been reported from other parts of Iran at different dates. For example, an occurrence of a maximum number of aphids was reported in August from Karaj (near the capital of Iran) (Rasoulian 1985). Another study showed a population peak for this aphid species in June (Monajemi and Esmaili 1981; Rasoulian 1985). Rakhshani *et al*. (2009) recorded two peaks for *A. pisum*, the second peak prominent in September−October 2004 and 2005. A high density of this aphid species was reported in June from Hokaido, Japan (Nakashi and Akashi 2005) and in June–July and again in November (Takahashi and Natio 1984).

The current findings of population fluctuations of lady beetles are concurrent with previous studies (Summers 1976; Wheeler 1977; Nakashima and Akashi 2005). Summers (1976) and Wheeler (1977) observed that populations of lady beetles in an alfalfa field in California and New York were high in May and September. More recently, a noticeable increase in a *H. variegata* population in early autumn was reported by Rebolledo *et al*. (2009) from Chile. As in the present study, Rakhshani *et al*. (2009) reported the peak of *H. variegata* on July 21 in the province of Isfahan (Central region of Iran), and their study confirms our results about the higher level of *H. variegata*. They found little significant correlation between the pea aphid population and Coccinellid predators or meteorological parameters.

The parasitoid wasp, *Dinocampus coccinellae* was collected in the alfalfa fields of studied region (Tavoosi Ajvad *et al*. 2012, 2014). This wasp frequently attacks to different lady beetle species including *H. variegata* and *C. septempunctata*.

The seven spotted lady beetle is a highly suitable host for *D. coccinellae* (Obrycki 1989; Geoghegan *et al*. 1997, 1998). Varied rates of *D. coccinellae* parasitism have been recorded for different lady beetle species. For example in the Firlej *et al*. (2005) study, the parasitism rate was reported as high as 32% in *Coleomegilla maculata*. Bjørnson (2008) reported 8% mean parasitism rate in *Hippodamia convergens* ranging from 3 to 15%, which was close to the rates obtained in the current study. Berkvens *et al*. (2010) recorded a parasitism rate from 0 to 14.7% for *Harmonia axyridis* Pallas. This result is inconsistent with that of Rebolledo (2009) and Tavoosi Ajvad *et al.* (2012) who both reported around a 30% overall mean parasitism rate in *H. variegata*. In the first year of our study, the mean parasitism rate on *C. septempunctata* was higher and it was expected because *D. coccinellae* prefers larger species (Hodek 1996; Ceryngier and Hodek 1996).

Several host characteristics influence successful parasitism by *D. coccinellae* including host stage, species (Geoghegan *et al*. 1998; Okuda and Ceryngier 2000), adult age, developmental stage (Majerus *et al*. 2000; Bjornson 2008), and sex (Majerus *et al*. 2000; Davis *et al*. 2006). Hodek and Honek (1996) summarised that survival of attacked lady beetles prior to the time of emergence of the *D. coccinellae* adult, was lower than that of non-attacked ones. Lady beetle females are more prone to be parasitised by this wasp because females are often larger than males. Another possibility might be that the parasitoid homes in on the combined scent of females and eggs, or just that the female is not moving much while laying eggs, thus giving the parasite a greater chance to deposit her eggs (James Grasela – personal communications). In our study, because of the different sizes of the attacked Coccinellids we expected the rate of *C. septempunctata* parasitism to be higher, and indeed, this was what was observed during the second year.

Although many factors influence pea aphid population density, the cutting and harvesting frequencies in the alfalfa ecosystem should be considered because they play a major role in aphid and lady beetle numbers. The population of lady beetles greatly oscillated across the years and sampling dates. These differences stress the fact that there is considerable variation in the parasitism of *D. coccinellae* and there is variation also in the effect of the meteorological factor influencing natural enemies, depending on the localities and the seasons sampled. Although, lady beetles might have contributed to the integrated pest management and suppression of the pests to below economic damage, it is likely that the presence of *D. coccinellae* reduced the effect of these beneficial predators.

Some limitations have been encountered e.g. in this study the preadult stages of the lady beetles were not been sampled and recorded, though useful information was indeed provided. Still, considering the role of other factors (e.g. harvesting date, nearby crops) on lady beetle populations, might provide more insights. Further studies are needed to clarify the role of harvesting and clarify the role of other groups of natural enemies on the lady beetle abundances.

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