Population Abundance and Seasonal Activity of Zetzellia pourmirzai (Acari: Stigmaeidae) and Its Preys Cenopalpus irani and Bryobia rubrioculus (Acari: Tetranychidae) in Sprayed Apple Orchards of Kermanshah, Iran

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ABSTRACT

The population densities and spatial distribution patterns of Zetzellia pourmirzai Khanjani and Ueckermann and its preys Cenopalpus irani Dosse and Bryobia rubrioculus (Scheuten) were studied in a sprayed orchard in Kermanshah, a western province of Iran, from 31 May till 7 November, 2007. The interaction (density dependence) between the plant feeding mites and their predator was determined as well. Population density of the phytophagous mites and their predator were counted on 130 leaves (sampling unit) every 10 days. The mean population density of C. irani per leaf was significantly higher than that of the other mites. The population densities of C. irani, B. rubrioculus, and Z. pourmirzai were highest on 9 August (11.092 per leaf), 20 July (0.554 per leaf) and 30 July (1.385 per leaf), respectively. The index of dispersion, regression models (Taylor and Iwao), and Lloyd's mean crowding to mean showed an aggregated distribution for all species. Some changes in the distribution from aggregated to random was indicated by Morisita's index during different sampling dates. These changes showed that the spatial distribution of these mites can vary during the season. The smallest optimum sample sizes, calculated with Taylors' coefficients, were 20.806, 192.912, and 128.117 for C. irani, B. rubrioculus and Z. pourmirzai, respectively. Linear regression of predator to prey population densities showed a density-dependant predation by Z. pourmirzai on C. irani and on B. rubrioculus. In addition, a significant linear regression was obtained between temperature and the population fluctuations of these mites. The spatial distribution parameters of the tetranychoid mites and their predator could be used to improve sampling programs and to estimate the population densities of these mites and the efficacy of the predator being used in orchards IPM.

Keywords: Bryobia rubrioculus, Cenopalpus irani, Population density, Spatial distribution, Zetzellia pourmirzai.

INTRODUCTION

Cosmopolitan mites of the superfamily Tetranychoidea can build up high population levels in some perennial agro-ecosystems like orchards (Duso *et al.*, 2004). A current control method for these pests is using acaricides on calendar based programs (Greco *et al.*, 2005), resulting in pest resistance and residue on products (Escudero and Ferragut, 2004). *Cenopalpus irani* Dosse and *Bryobia rubrioculus* Scheuten have been found in large numbers in apple orchards of Iran (Rashki *et al.*, 2004; Darbemamieh, 2008). Orchard management practices usually cause outbreaks of spider mites followed by disrupted population of natural enemy or induce mite migration from the ground cover into trees (Alston, 1994). Biological control is a useful alternative to pesticides for managing

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various arthropod pests (Opit *et al.*, 2005) and the predator mites are one of the most important factors in reducing tetranychoids and other plant feeding mite populations (Gerson *et al.*, 2003).

The possible influence of stigmaeids in commercial agricultural systems is not well known and is usually considered to be minor (Villanueva and Harmsen, 1998). Stigmaeids live on plants or in the soil and feed on tetranychids, tenuipalpids or eriophyids (Kheradmand et al., 2007). Zetzellia mali (Ewing), one of the most important predator species of Stigmaeidae, has wide distribution in apple orchards (Croft and Slone, 1997). It can feed on eggs and immature stages of B. rubrioculus (Croft & Slone, 1997) and C. irani. Zetzellia pourmirzai Khanjani and Ueckermann, a related species to Z. mali, has found in Kermanshah province been (Khanjani & Ueckermann, 2008). This species has been considered as a predator foraging with the same regimes (Darbemamieh, 2008). Zetzellia spp. Possess a desirable predator attribute, which is the ability of surviving for long periods of time with low density of prey (Villanueva and Harmsen, 1998) that can be noticeable in sprayed ecosystems. The role of Z. pourmirzai as predator of various tetranychid and tenuipalpid mites in apple orchards needs to be studied carefully.

Having information about spatial distribution of prey and predator is critical in evaluating the natural enemy potential to reduce its prey density and increase the system persistence (Slone and Croft, 1998). It can also be used to investigate population dispersion behavior, establish a precise sampling plan, and program sequential sampling (Athanassiou et al., 2003 a and b, 2005 a and b; Kavallieratos et al., 2002, 2004 and 2005; Deligeorgidis et al., 2002; Margolis et al., 1984), detect pest levels for justifying control measures (Arnaldo and Torres, 2005; Athanassiou et al., 2003 a and b, 2005; Kavallieratos et al., 2002 and 2005), and assess crop loss (Hughes, 1996). Spatial distributions of C. irani, B. rubrioculus and Z. mali have been studied in an unsprayed

orchard of the region (Darbemamieh, 2008). Changes in the distribution pattern in sprayed condition could be another topic for study.

Using information of sample mean, variance and size, the variance-mean relationships of Taylor (1961) and Iwao (1968) have been effectively used in many sampling procedures (Beers and Jones, 2004; Hamilton and Hepworth, 2004; Kavallieratos et al., 2002). Quantitative knowledge of spatial distribution patterns of plant feeding mites and their natural enemies is essential for understanding their interactions and developing reliable sampling plans for monitoring pest and predator abundance (Onzo et al., 2005; Athanassiou et al., 2005a).

Since there are few studies about spatial distribution of tenuipalpids and stigmaeids and no study about *Z. pourmirzai*, the results of this study can be used as a base for monitoring and controlling of mites for establishing IPM strategies in sprayed apple orchards. The calculated common coefficients of this study could be used for various controlled apple cultivars and similar orchards.

MATERIALS AND METHODS

Sampling Procedure

In this study, one apple leaf was selected as a sample unit. Leaves were selected randomly and from all parts of the canopy, avoiding biased estimate of population mean. Samples were taken at 9-12 A.M. from 31 May till 7 November, 2007, in 10day intervals. Each leaf was placed in a separate zip-kip nylon pocket and kept in a portable flask at 4°C during transport process. In the laboratory, the number of motile stages of C. irani, B. rubrioculus, and their predator, Z. pourmirzai, was counted leaf at the same day using per stereomicroscope (Olympus, made in Japan.). After primary sampling, the relative variation (RV) was calculated according to Hillhoxuse and Pitre (1974) to evaluate the efficiency of the data.

Spatial Distribution

The spatial distribution of *Z. pourmirzai* and its preys was determined by the following five methods: index of dispersion, Morisita's coefficient of dispersion, Lloyd's mean crowding, and regression techniques of Taylor's Power Law and Iwao's Patchiness.

Index of dispersion

Dispersion of a population can be classified by calculating the variance (S^2) to mean (m) ratio as follows: $S^2/m>1$ is classified as Aggregated Distribution, while $S^2/m = 1$ or <1 are classified as Random or Regular Distribution, respectively. Departure from a random distribution can be tested by calculating the index of dispersion, I_D , as in Eq.1:

$$I_{\rm D} = (n-1)S^2 / m \ (1)$$

Where n is the number of samples. In order to test the goodness of fit, Z coefficient should be calculated according to Eq.2 shown below:

$$Z = \sqrt{2 I_{D}} - \sqrt{(2 v - 1)}$$
(2)

Where v is degrees of freedom (*n*-1) (Patil and Stiteler, 1974).

Regression techniques

According to Taylor's power law, population variance (S^2) is proportional to a fractional power of the arithmetic mean (m) as in Eq. 3 (Taylor, 1961):

$$\log S^2 = \log a + b \log m \tag{3}$$

Iwao's patchiness regression method quantifies the relationship between mean crowding index (m^*) and mean (m) using Eq. 4 (Iwao, 1968):

$$m^* = \alpha + \beta m$$

Student t-test can be used to determine whether the colonies are randomly dispersed.

Calculated values are compared with tabulated t-values with n-2 degrees of freedom.

Morisita's coefficient of dispersion (I_{δ})

Morisita (1962) proposed a hypothesis for testing the uneven distribution coefficient of I_{δ} , which is calculated using Eq. 5 (Pedigo and Buntin, 1994):

$$I_{\delta} = \frac{n \sum x_i(x_i - 1)}{N(N - 1)} \tag{5}$$

Equation 6 can be used to determine whether the sampled population significantly differs from the random distribution:

$$z = \frac{\left(I_{\delta} - 1\right)}{\left(\frac{2}{nm^2}\right)^{\frac{1}{2}}} \tag{6}$$

Lloyd's mean crowding (x^{T})

Theoretically, mean crowding (x^*) is the mean number of other individuals per individual in the same quadrate as given by Eq.7 (Lloyd, 1967):

$$x^* = m + \frac{s^2}{m} - 1 \tag{7}$$

Optimum Number of Sampling Units

Finding out the generic coefficients eliminates experimental needs for large sample size (Ifoulis and Savopoulou-Soultani, 2006). The optimum sample size, i.e., the smallest number of sample units

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(4

with precision of estimates is calculated by using coefficients a and b within Taylor's power law in Eq. 8:

$$N_{opt} = a \left(\frac{t_{\alpha/2}}{D}\right)^2 \left(\mu^{b-2}\right)$$
(8)

Where N _{opt} = sample size, $t_{\alpha/2}$ = tstudent of table, μ = mean density, a and b = Taylor's coefficients and D = the range of accuracy.

The optimum sample size is derived from Eq. 9:

$$N_{opt} = \left(\frac{t_{\alpha/2}}{D}\right)^2 \left(\frac{1}{\mu} + \frac{1}{k}\right)$$
(9)

Where k in negative binomial distribution is given in Eq.10:

$$\frac{1}{k} = \frac{\sigma^2 - \mu}{\mu^2} \tag{10}$$

This estimation can also be done by Iwao's patchiness regression method coefficients (α and β) in Eq. 11(Wilson, 1985):

$$N_{opt} = \left(\frac{t_{\alpha/2}}{D}\right)^2 \left(\frac{\alpha+1}{\mu} + (\beta-1)\right)$$
(11)

Correlation coefficient values were tested for departure from zero, by using the twotailed t test, at n-2 degrees of freedom.

Density Dependence in Prey-Predator Interaction

Type of interaction between prey and

predator is determined by using simple linear regression between the prey and the predator population densities. Predator would be density independent in case of *P*-value > 0.05 (b = 0), but if *P*-value ≤ 0.05 and b > 0 or b < 0, predator would act as density dependent and inverse density dependent in its predation activity, respectively.

Effect of Temperature and Humidity

Linear regression between temperature (°C) or humidity (RH%) and population density in each date have been used to determine the effect of these two factors on population fluctuation of mites. High values of *r* and *P*-value ≤ 0.05 can be an indicator for positive relations and no relation in case of *P*-value > 0.05 (Table2).The daily records of the temperature and humidity were obtained from Kermanshah Meteorological Station, located10 km away from the orchard.

RESULTS AND DISCUSSION

Population Fluctuation

Data set from primary sampling were used to calculate RV. The biggest calculated RVand reliable sample size were 6.66% and 130, respectively. Population fluctuations of

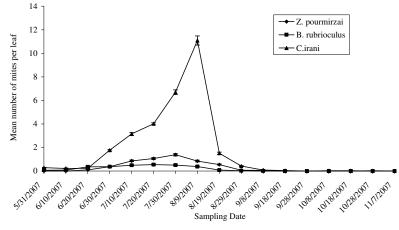


Figure 1. Population fluctuation of *Cenopalpus irani*, *Zetzellia pourmirzai*, and *Bryobia rubrioculus* on apple leaves in 2007.

Table1. Estimated values of intercept and slope of linear regression analysis between temperature or humidity and population density for *Cenopalpus irani, Bryobia rubrioculus* and *Zetzellia pourmirzai* in 2007.

a .	Temperature				Humidity			
Species	а	b	r^2	Pvalue	α	b	r^2	Pvalue
C. irani	-7.378	0.374	0.401	0.006	0.380	-0.151	0.173	0.096
Z. pourmirzai	- 1.216	0.063	0.513	0.001	0.842	-0.022	0.163	0.108
B. rubrioculus	-0.624	0.033	0.624	0.000	0.385	-0.009	0.120	0.173

C. irani, B. rubrioculus and Z. pourmirzai are shown separately (Figure 1). Both C. irani and B. rubrioculus were observed from the beginning of the sampling period (31 May), but no individuals of Z. pourmirzai were recorded before 20 June. The first observation of C. irani was made by the end of May, but its population didn't increase till 20 June, peaking on 9 August, 2007 (11.092 per leaf). The population density of C. irani increased in late July and early August as a result of increase in temperature and dryness. Furthermore, the sharp decline in C. irani population that was observed from mid August till mid September may have been due to the predator's activity. Meanwhile, the peak of Z. pourmirzai population was observed in the end of July (1.385 per leaf). Since the predator feeds on eggs, the resulting decrease in prey population through the next generation could be the probable reason for the gap between peaks of predator and prey populations. Decrease in population of Z. pourmirzai may be in part due to the severe decrease in C. irani population at the end of summer. The population of *C. irani* could not surely increase again due to the lower temperature after September (Figures 1 and 2). Population density of *C. irani* per sample unit was higher than *B. rubrioculus* and also remained in longer duration, suggesting that *C. irani* might be the most abundant and serious acari pest in apple orchards of the region.

The highest population density of *B. rubrioculus* was in 20 July (0. 554 per leaf). Population of *B. rubrioculus* is much more sensitive to predation in comparison to *C. irani* and could be readily controlled because of the bigger size of the adults and the smallest capacity for population increase (Darbemamieh *et al.*, 2009). It seems that other predator species can affect the population of *B. rubrioculus*, too. Therefore, in low population density of this pest, the egg predator can easily keep its population in low density.

Linear regressions between temperature and population densities of the three studied

Table 2. Estimated values of intercept and slope for *Cenopalpus irani, Bryobia rubrioculus* and *Zetzellia pourmirzai* in 2007 by regression analysis of Taylor's Power Law and Iwao's Patchiness Regression (t test for goodness of fit).

	Taylor's power law						
	а	b	r^2	P-value			
C. irani	0.364±0.066	1.126±0.076	0.952	0.000**			
Z. pourmirzai	0.502 ± 0.045	1.130±0.044	0.985	0.000**			
B. rubrioculus	0.511±0.067	1.225±0.065	0.975	0.000**			
		Iwao's patchine	ess regression				
	а	b	r^2	P-value			
C. irani	0.761±0.433	1.341±0.110	0.931	0.000**			
Z. pourmirzai	1.106±0.212	2.195±0.329	0.817	0.000**			
B. rubrioculus	0.612±0.387	3.494±1.155	0.504	0.014*			

* Significant at $\alpha = 0.05$

** Significant at $\alpha = 0.01$

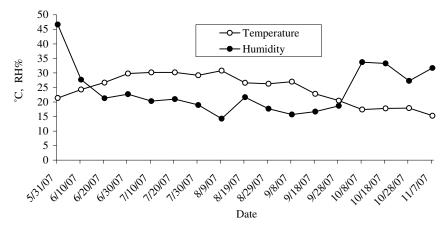


Figure2. Temperature and humidity records of Kermanshah Meteorological Station during the sampling period.

mites were significant. That, in turn, confirmed the great effect of temperature on population fluctuations of these mites (Figures 1, 2, and Table 1). No significant regression was observed between humidity and population density of mites. These observations are consistent with the reported data in unsprayed orchards (Darbemamieh, 2008). Similar observations have been reported from the central parts of Iran (Nowzari, 1992). It can be concluded that temperature plays an important role in population fluctuations of plant feeding mites in the studied region.

Spatial Distribution

Both regression methods fit the data well for all the examined species (Table 2). The results of Taylor and Iwao regression methods showed that the spatial distribution pattern of false spider mite, brown spider mite, and their predator were aggregated. Data demonstrated good fitness to both Taylor's ($r^2 = 0.952$) and Iwao's model ($r^2 = 0.931$) for *C. irani*, and also better fitness with Taylor's model for *B. rubrioculus* ($r^2 = 0.975$) and *Z. pourmirzai* ($r^2 = 0.985$), when compared to Iwao's model. Taylor's power law as well as Iowa's patchiness regression has been widely used for evaluating dispersion, data normalizing for statistical analysis, and sampling protocols for many insects (Davis, 1994 ; Deligeorgidis *et al.*, 2002).

The index of dispersion (I_D) and the m^*/m values for all populations were significantly greater than 1 (Table 3), which means these species exhibited aggregated behavior in their habitat. Observed aggregation in spatial distribution for all examined species with the index of dispersion and Lloyd mean crowding suggests that presence of an individual mite at one point may cause increased probability of another individual being nearby. In addition, probability of habitat occupation by individuals would not be the same among different species. Based on these results, population distribution of

Table 3. Estimated parameters by Lloyd mean crowding, index of dispersion, Lloyd mean crowding to mean and common *k* for *Cenopalpus irani*, *Bryobia rubrioculus* and *Zetzellia pourmirzai* in 2007.

Species	т	S^2	m^*	I_D	Ζ	m [*] /m	1/k
C. irani	2.682	21.829	9.822	11639.886	99.117	3.662	2.662
B. rubrioculus	0.267	0.953	2.064	4000.641	35.990	7.729	6.729
Z. pourmirzai	0.484	0.747	3.138	5225.903	48.774	6.485	5.485

the predator is tightly linked to the prey distribution, a characteristic that would create refuges for the prey and, consequently, increase the persistence of the system.

Comparing 1/k values among the three species showed that the aggregation of B. rubrioculus was bigger than that of the other species because of higher value of the 1/kindex. This might be due to bigger differences between the variance and mean of this mite sampling data. All of the 1/kcalculated suggests aggregated values pattern of dispersion for these three species. Aggregated distribution of spider mites has been shown in other studies too (Nuchman, 1984; Strong et al., 1997). It seems that dispersion indexes are convenient decision making factors for management programs because of their easy calculation procedure and simple results.

There were some differences in Morisita's index values of each species, but, in most sampling dates, the index was significantly greater than 1.96 (Table 4), suggesting that the spatial distribution of all species was aggregated. Since Morisita's coefficient estimates spatial distribution using the mean and variance of each sampling date

separately, this index is more accurate than index. the dispersion Showing one distribution per each date can be used to understand details of dispersion in different sampling dates that would be useful for research strategies more than management programs. Changes in the distribution of C. irani in late June, July, and early August from aggregated to random can be partly due to the increase in population density or movement of nymphs from the clumped egg locations. It shows that distribution pattern can change as the result of some factors during the sampling season. It seems that distribution pattern in most of the sampling dates could be used as a base for management decisions. Although Taylor's indices have been widely used by many researchers, we suggest Morisita's index for research programs because of its higher determination coefficient as well as better dispersion interpretation for these species. Spatial distribution of the studied mites using different analytical methods showed aggregated or random pattern, suggesting that the different statistical methods have various accuracies in calculating spatial distribution of an organism.

The spatial distribution of population

Table 4. Morisita's index and Z values for *Cenopalpus irani, Bryobia rubrioculus* and *Zetzellia pourmirzai* in different sampling dates of 2007 (Z test for goodness of fit).

Species	C.irani		Z. pourmi	rzai	B.rubrio	culus
Date	I_{δ}	z	I_{δ}	Z	I_{δ}	z
31-May	4.68	104.37	-	-	18.05	1986.20
10-Jun	17.29	588.76	-	-	13.79	116.91
20-Jun	5.03	136.33	15.00	1128.72	9.42	191.85
30-Jun	1.28	1.27	6.97	133.24	6.57	121.60
10-Jul	1.371	0.947	3.123	19.688	3.55	41.705
20-Jul	0.995	-0.010	3.795	21.232	3.86	41.71
30-Jul	1.31	0.38	2.67	9.73	2.91	30.32
9-Aug	1.54	0.39	3.68	25.34	7.32	132.53
19-Aug	2.33	7.07	4.02	43.93	2.36	129.93
29-Aug	3.59	49.34	5.78	500.76	43.33	14789.76
8-Sep	16.54	1481.19	23.21	2910.33	-	-
18-Sep	13.00	2515.42	-	-	-	-
28-Sep	-	-	-	-	-	-
8-Oct	-	-	130.00	45068.02	-	-
18-Oct	-	-	43.33	14789.76	-	-
28-Oct	0.00	-349.36	-	-	-	-
7-Nov	-	-	130.00	67602.03	-	-



individuals in an ecosystem can be due to behavioral patterns and environment. Despite parameters such as rate of population increase and reproduction that change from one generation to another, spatial distribution is partially constant and is a characteristic of the species (Taylor, 1984). Clump laying behavior and slow movement of C. irani, B. rubrioculus and Z. pourmirzai could be accounted as possible for their aggregated reasons spatial distribution. The spatial distribution of a prey can determine the spatial distribution of its natural enemy. For example, searching rate of phytoseiids in aggregated populations of spider mites is higher than that observed in tetranychid populations with random distribution (Kim and Lee, 1993).

Optimum Number of Sample Units

In this study, the absolute counts of *Cenopalpus irani*, *Bryobia rubrioculus* and *Zetzellia pourmirzai* motile stages were used to develop generic coefficients of regression techniques based on large amount of data. The sample size was re-calculated using k in negative binomial distribution and Taylor's and Iwao's coefficient, i.e. a, b, α and β (Table 5). The lowest estimate of the sample size was calculated by using Taylor's equation, for all species.

In comparative studies of models, Taylor's power law model usually showed to fit the spatial dispersion better than Iwao's model to achieve a desired precision of estimates (Afshari *et al.*, 2009). Basically, Taylor's method reduces the necessary sample size by almost half compared to the common k or Iwao's method. The latter was originally

derived with close reference to the theoretical distribution models (Davis. 1994). This may account for the observed similarity with the calculated amounts using the common k and Iwao's method. In contrast, as a purely empirical model, Taylor's power law doesn't have such definite theoretical basis (Kuno, 1991); however, it has been widely used because of its statistical stability. In this study, in order to acquire greater precision, we adopted the 20% level, whereas in IPM programs 25% or 30% level is acceptable. Optimal sample size suggested by Taylor's model is typically higher at low population levels. Athanassiou et al., (2005b) also suggest a sampling plan based on Taylor's estimates, for Myzus persicae and Macrolophus costalis.

The optimum sample size calculated at the beginning of the study was useful for all species, except *B. rubrioculus*. The lower population density of this mite might have led to large sample size. Seemingly, using Taylor's power law coefficients suggests lower actual sample sizes and could lead to better results in situations like the present study.

Density Dependence in Prey-Predator Interaction

The linear regression coefficient between population densities of *C. irani* and *Z. pourmirzai* was statistically significant ($r^2 = 0.660$, P< 0.000) as well as for *B. rubrioculus* and *Z. pourmirzai* ($r^2 = 0.744$, P< 0.000). This suggests close relation and statistically significant linear regression between these species fluctuations. The non-linear response of *Z. mali* to *Tetranychus*

Table 5. Calculated sample size of *Cenopalpus irani, Bryobia rubrioculus* and *Zetzellia pourmirzai* populations on apple leaves based on k in negative binomial distribution and Taylor's power law and Iwao's patchiness coefficients in 2007.

	Species	C. irani	B. rubrioculus	Z. pourmirzai
n _{opt}				
Κ		411.941	1421.406	1024.96
Taylor		20.806	192.912	128.117
Iwao		135.403	1157.503	752.852

turkestani (Ugarov and Nikolskii) density has been previously revealed (Khodayari, 2007); however, population fluctuation curves showed delayed density-dependent response of predator to its prey density. Lawson and Walde (1993) reported that Z. mali, which has been thought to be less important in the control of Panonychus ulmi (Koch), exhibits a stronger response to the prey density than T. pyri. The significant linear regression model between prey and predator densities in our study suggested dependent predation density by Ζ. pourmirzai on its two prey species in the sprayed apple orchards. Similar results have been reported for Z. mali in an unsprayed orchard of this region (Darbemamieh, 2008). The density-dependent predation by Z. pourmirzai on its two preys does not mean that this predator is capable of controlling these phytophagous mites without help. Therefore, it will be necessary to study the r_m (intrinsic rate of natural increase) of this predator when it feeds on each of the mites, namely, B. rubrioculus and C. irani, and determine how much it can eat daily.

As a conclusion, our study shows that the spatial distribution indices of the Z. *pourmirzai* and its two preys can be used in sampling programs as well as in proper estimation of their population densities in protection management of apple orchards.

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فراوانی جمعیت و فعالیت فصلی کنه شکار گر (Acari: Stigmaeidae) فراوانی جمعیت و فعالیت فصلی کنه شکار گر و طعمه های آن *Cenopalpus irani* و (Acari: Tetranychidae) و Bryobia rubrioculus (Acari: Tetranychidae) باغات سمیاشی شده سیب منطقه کرمانشاه

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تراکم جمعیت و الگوی توزیع فضایی کنه شکار گر & Cenopalpus irani Dosse و Bryobia و Bryobia و Cenopalpus irani Dosse و Bryobia در یک باغ سمپاشی شده در کرمانشاه از ۱۱/ ۳/ ۱۳۸۶ تا ۱۴/ ۸/ ۱۳۸۶ بررسی شد. همچنین نوع وابستگی تغذیه شکار گر به تراکم جمعیت کنههای گیاه خوار به عنوان یکی از ملاکهای ارزیابی کارایی شکار گر مشخص گردید. جمعیت کنههای گیاه خوار و شکار گر روی ۱۳۰ برگن واحد نمونه برداری) هر ۱۰ روز یک بار شمارش شد. برای تعیین الگوی توزیع فضایی گونه ها از