Seasonal Activity of Zetzellia mali (Stigmaeidae) and Its Preys Eotetranychus frosti (Tetranychidae) and Tydeus longisetosus (Tydeidae) in Unsprayed Apple Orchards of Maragheh, Northwestern of Iran

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ABSTRACT

Seasonal activity and spatial distribution of *Eoteranychus frosti* Mcgregor, *Tydeus longisetosus* (ElBagouy and Momen) and *Zetzellia mali* Ewing were studied during 9th July to 22th October 2006 in an unsprayed apple orchard, Maragheh region, Northwest of Iran. Also the density dependence interaction between preys and predator was assessed. To estimate the spatial distribution pattern of these species, data was analyzed through Iwao's patchiness regression, Taylor's power law, Morisita's coefficient, Lloyd's mean crowding and index of dispersion. Iwao's patchiness regressions and Taylor's power law showed a random and Morisita's coefficient, Lloyd's mean crowding and index of dispersion revealed an aggregated distribution pattern for *E. frosti, T. longisetosus* and *Z. mali.* The results indicated that the peak density of *E. frosti* and *T. longisetosus* occurred in mid September and in late August (2.46 and 4.4 per leaf, respectively). Regarding their predator, the peak density (10.34) occurred in early July. The linear regression between prey and predator densities indicated a density independent predation by *Z. mali.*

Keywords: Seasonal activity, Spatial distribution, *Eoteranychus frosti, Tydeus longisetosus*, *Zetzellia mali.*

INTRODUCTION

Mites of the superfamilies Tydeoidea and Tetranychoidea are cosmopolitan, commonly distributed in all continents and climatic zones of the world. They are widely distributed and build up high population levels in some perennial agro-ecosystems (Duso et al., 2005). Spider mites (Acari: Tetranychidae), being very harmful are widespread pests throughout apple growing areas (Kasap, 2005). They ingest leaf cell contents, thus reducing plant photosynthesis, potentially decreasing fruit quality (Prischmann et al., 2005). Unless controlled,

these mites may cause severe damage to crop quantity and to quality as well (Kasap, 2005). A current control method of this pest is to use acaricides on a calendar based (Greco et al., 2005), with the resulting problems of pest resistance and residue on the harvested and then consumed products (Escudero and Ferragut, 2005). To reduce pesticide input and its associated risks as well as costs, biological control of spider mites is widely used worldwide (Mo and Liu, 2006).

Predaceous mites are important natural enemies of several such phytophagous mites such as *Tetranychus urticae* Koch and other pests of various crops and are known to play

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an important role in the natural control of these pests (Kasap, 2005). The main predatory mites in apple orchards belong to the families Phytoseiidae and Stigmaeidae. Despite the role of phytoseiid mites having been broadly studied, the role of stigmaeids in commercial agricultural systems is not well known and has usually been considered to be minor (Villanueva and Harmsen, 1998). To fill this gap, an investigation was carried out on a reliable and scientific sampling program of, spatial distribution and seasonal activity of Zetzellia mali Ewing and its two preys, Eotetranychus frosti McGregor (Acari: Tetranychidae) and Tydeus longisetosus (ElBagouy and Momen)(Acari: Tydeidae) in an unsprayed apple orchard, Maragheh region, Iran.

Estimating the population density of arthropods is the cornerstone of basic research on agricultural ecosystems and the principal tool for building and implementing pest management programs (Kogan and Herzog, 1980). A knowledge of spatial distribution of preys and of predators is indispensable to evaluate the system persistence and as well the potential of a natural enemy to reduce its prey density (Slone and Croft, 1998b). Field distribution of a pest is an important determinant of the number of samples required for its population estimate. Spatial distribution is a tool to used in crop loss assessment (Haughes, 1996), ecological and behavioral investigations (Faleiro et al., 2002) as well as in population growth determination (Jarosik et al., 2003). Spatial distribution of T. urticae was studied by Shih and Wang (1996) in Carambola Orchard. Croft and Slone (1997) studied equilibrium on densities of European red mite after exposure to three levels of predaceous mite diversity on apple. Villanueva and Harmsen (1998) investigated the role of predatory mite Z. mali in an experimental apple orchard which was used primarily for the elevation of a new IPM protocol for the combined control of the spotted tentiform leaf miner as well as spider mites. Slone and Croft (1998a) described aggregation for 8 mite species in apple orchards including the plant feeding spider mites, *T. urticae* + *Eotetranychus* sp., *Panonychus ulmi* Koch and *Bryobia rubrioculus* (Scheuten) and the stigmaeid predator *Z. mali*, phytoseiid predators *Amblyseius andersoni* Chant, *Metaseiulus occidentalis* (Nesbitt) and *Typhlodromus pyri* Scheuten. They also reported that *Z. mali* shows significant increase in aggregation when other predators were present. Greco et al. (1999) studied on spatial distribution and coincidence of *Neoseiulus californicus* (McGegor) and *T.*

urticae on strawberry.

Seasonal cycle and population dynamics of Schisotetranychus nanjingensis (Ma and Yuan), Aponychus *corpuzae* Rimando (Acari: Tetranychidae), Aculus bambusae Kuang (Acari: Eriophyidae) and their natural Typhlodromus bambusae Ehara enemv (Acari: Phytoseiidae) were studied by Zhang et al. (2001) in moso bamboo forests of China. Duso et al. (2005) investigated the effects of grape Downey mildew spread on seasonal abundance of Tydeus caudatus Duges and its predators. So (2006) studied on distribution patterns and sampling of T. urticae on roses.

Interactions between predator and prey were expected to be mixed with the variation of responses not seen as a great surprise. A likely characteristic of an efficient specialist predator is its high searching capacity for its preferred food item (Slone and Croft, 2001). Responses of two predaceous mites, *T. pyri* and *Z. mali* to variation in prey density were studied by Lawson and Walde (1993). Slone and Croft (2001) investigated on species association among predaceous and phytophagous apple mites.

In our present study on phytophagous mites and their natural enemies in an unsprayed apple orchard of Maragheh (East Azarbayjan province,Iran), three major species, *E. frosti, T. longisetosus* and *Z. mali* were the most abundant. The goal of this research was to investigate seasonal activity and spatial distribution of *E. frosti, T. longisetosus* and *Z. mali* and the density dependence interaction between predator and its preys, *E. frosti* and *T. longisetsus*. The results of this study can be employed as basic information to develop and optimize reliable sampling plans, monitoring methods and control of these pests for establishing IPM strategies.

MATERIALS AND METHODS

Sampling Program

Sampling unit: Different life stages of *Z. mali* and its preys, *E. frosti* and *T. longisetsus* usually colonize on the undersurface of leaves, thus a leaf was selected as sampling unit. Sampling of the leaves was randomly carried out and counted number of the mites was carried out under the dissection stereomicroscope in the laboratory.

Pattern and timing of sampling: Sampling of leaves and the movement among trees were randomly performed. From 9th July to 22th October 2006, samples were taken weekly (11 times) in the mornings from about 30 apple trees (5 leaves per tree, taken from the middle height). The orchard had not been sprayed for more than 10 years.

Sample size: To determine the number of samples it is necessary to have random primary sampling to calculate RV (Relative Variation). Sample size can be determined if RV value is less than 25% (Pedigo and Buntin, 1994). Relative variance is calculated as follows:

$$RV = (\frac{SE}{m})100$$

Where SE = Standard Error and m = Mean density of primary sampling data

In case RV value was greater than 25%, more samples should be taken in the primary sampling. Sample size formula is as follows:

$$N = \left(\frac{t \times s}{d \times m}\right)^2$$

where s = Standard deviation, m = Mean density of primary sampling data, t = Standard normal variance for two-tailed interval and d = Range of accuracy

Spatial Distribution

Iwao's patchiness regression and :The distribution Taylor's power law pattern of three species were analyzed by methods of Iwao (1968) and Taylor (1961). showed that Iwao mean crowding $\left(s^{2}\right)$)

$$m^* = \overline{x} + \left(\frac{\overline{x}}{m} - 1\right)$$
 and mean density (m)

exhibit a linear relationship in many populations:

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

where α is the number of other individuals with which an individual shares the sampling unit and β is the measure of the distribution of populations, where β takes values of 1, <1, and >1 for random, uniform and aggregated distributions, respectively.

Taylor's power law $s^2 = am^b$ relates variance (s²) to mean density (*m*). We can solve for the coefficients with linear regression if a log transformation (base 10 or natural log) is employed:

 $\log s^2 = \log a + b \log m$

The coefficient *a* has been described as a scaling factor related to sample size and *b* measures the species aggregation. If b=1, <1 and >1 the distribution is random, uniform and aggregated respectively, with aggregation increasing with increases in b. Student *t*-test can be employed to determine if the colonies are randomly dispersed.

Test b = 1 $t = (b-1)/s_b$ and Test $\beta = 1$

$$t = (\beta - 1) / s_{\beta}$$

Where s_b and s_β are the standard error of the slope for the mean crowding regression. Calculated values are compared with the tabulated *t*-values within n-2 degrees of freedom.

Index of dispersion: Dispersion of a population can be classified by calculating

the variance to mean ratio: $s^2 / m = 1$ random, <1 regular while>1 aggregated. Departure from a random distribution can be tested by calculating the index of dispersion (I_D) , where *n* is the number of samples:

$$I_D = (n-1)s^2 / m$$

 I_D is approximately distributed as χ^2 with

n-1 degrees of freedom. Values of I_D which fall outside a confidence interval bounded by χ^2 with *n*-1 degrees of freedom and selected probability levels of 0.95 and 0.05, for instance, would indicate a significant departure from a random distribution. This index can be tested by z value as follow:

$$z = \sqrt{2\chi^2} - \sqrt{2\nu - 1}$$
$$\nu = n - 1$$

If $1.96 \ge z \ge -1.96$ the spatial distribution would be random but if z < -1.96 and z > 1.96 it would be uniform and aggregated, respectively.

Morisita's coefficient of dispersion I_{δ} : Morisita (1959) reported a hypothesis for testing the uneven distribution coefficient of I_{δ} which is calculated by the formula:

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

n = The number of sample units, x_i = The number of individuals in each sample unit and N = Total number of individuals in nsamples

To determine if the sampled population significantly differs from random, the following large sample test of significance can be used:

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

If $1.96 \ge z \ge -1.96$ the spatial distribution would be random but if z < -1.96 and z > 1.96 it would be uniform and aggregated, respectively.

Lloyd's mean crowding x^* : A third index, termed mean crowding (x^*) was

proposed by Lloyd (1967) to indicate the possible effect of mutual interference or competition among individuals. Theoretically, mean crowding is the mean number of other individuals per each individual in the same quadrate:

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

As an index, mean crowding is highly dependent upon both the degree of clumping and population density. To remove the effect of changes in density, Lloyd introduced the index of patchiness, expressed as the ratio of mean crowding to the mean. As with the variance-to- mean ratio, the index of patchiness is dependent upon quadrate size $x^*/m=1$ random, <1 regular and >1 aggregated (Pedigo and Buntin, 1994).

Sample size model: Taylor's a and b coefficients, taken from Taylor's power law describe the relationship between variance $(s^2 = am^b)$ for mean individuals and distributed in a natural population. The mean and variance of sampled mites were determined for each weekly sampling span of time (date). Taylor's a and b coefficient calculated were by log-log linear transformation of the mean-variance data, where b is the slope of the transformed data and a equals the antilog of transformed intercept. An equation for estimating pest sample size was developed by Karandinos. Ruesink, Wilson and Room incorporated Taylor's power law into Karandinos' equation to form the sample size model used in this study (Cullen et al., 2000):

$$N = t_{\alpha/2}^{2} d^{-2} a m^{b-2}$$

The model contains both variable and constant factors. The variable factors are:

N = Sample size, $t_{\alpha/2}$ = Standard normal variance for a two-tailed interval, m = Mean density of mites in each sampling unit, d = The range of accuracy and a, b = Taylor's coefficients.

Coefficient of aggregation: Many indices used for describing species aggregation are based on the mean and variance of samples.

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These include the negative binomial common k, Lloyd's index of mean crowding, as well as Iwao's patchiness regression. A simple method for calculating k is based upon the proportion of zeros obtained in samples. If N is the total number of samples and N_0 the number of samples with zero individuals, k would be estimated by using the following interactive equation (Pedigo and Buntin, 1994):

 $\log(N/N_0) = \hat{k}\log(1+m/\hat{k})$

Density dependence interaction: To determine the type of interaction between prey and predator, analysis of simple linear regression was carried out between prey and predator densities at different spans of time. If P-value >0.05 (b=0) predator would be density independent but if P-value is <0.05 and b>0 or b<0, predator would act as density dependent and inverse density dependent, respectively.

RESULTS

Sampling Program

Data sets from primary samplings were used to calculate RV. Calculated RV and sample size for *Z. mali* were 6 and 61.66, respectively.

Seasonal activity

Population fluctuation curves of *E. frosti*, *T. longisetosus* and *Z. mali* populations in order to determine seasonal activity are shown in Figure 1.

Spatial Distribution

Iwao's α and β and Taylor's *a* and *b* coefficients, *t*-calculated and *t*-table for each species are shown in Table 1. Accordingly, Iwao's and Taylor's regressions were highly significant and β and *b* values were equal to 1 because *t*-calculated<*t*-table.

Results of calculating index of dispersion (I_D) revealed that the spatial distribution of populations detected in apple orchards tended to be aggregated. Calculated I_D values for *E. frosti, T. longisetosus* and *Z. mali* were 3728.8, 3579.16 and 7500.59, respectively, significantly more than 1.

Morisita's index of all species had values significantly greater than 1.96 at all the sampling dates (Table 2).

Calculated Lloyd's mean crowding for *E. frosti, T. longisetosus* and *Z. mali* were 3.97, 4.23 and 9.78, respectively, indicating the aggregated spatial distribution of these species. Calculated sample size for *Z. mali* through sample size model formula was 27.21. Coefficient of aggregation was calculated for all species and, shown in Table 3.

There was not any significant relationship observed between stigmaeid-tetranychid and stigmaeid-tydeid densities as P-value of regression was recorded greater than 0.05,



Figure1. Population fluctuation of Z. mali, E. frosti and T. longisetosus during 2006.



Species	α	β	$\log a$	b	r^2	t _{cal}	t _{table}	SE	Р
Iwao's patchiness regression									
Eotetranychus frosti	0.73	2.22	-	-	0.504	1.76	2.66	0.69	0.013
Tydeus longisetosus	2.01	1.24	-	-	0.593	0.77	2.66	0.31	0.003
Zetzellia mali	0.919	1.43	-	-	0.889	2.86	2.66	0.15	0.000
Taylor's power law									
Eotetranychus frosti	-	-	0.482	1.31	0.88	1.93	2.66	0.16	0.000
Tydeus longisetosus	-	-	0.493	1.09	0.79	0.52	2.66	0.17	0.000
Zetzellia mali	-	-	0.387	1.27	0.93	2.45	2.66	0.11	0.000

Table 1. Estimated values of α , β and *a*, *b* of different species through Iwao's patchiness regression and Taylor's power law models.

suggesting that predator acts as density, independent of prey densities.

DISCUSSION

In developing sampling programs for either research or pest management purposes, one must determine two characteristics of any population, namely: its density and dispersion.

For an applied population biologist, knowledge of a population's aggregation is indispensable to develop sequential, binomial or other sampling plans. A change in aggregation of a species often requires an

Table 2. Morisita's index and z values for three species at different dates

Date	E. frosti	T. longisetosus	Z. mali	
9 Jul	N / A^a	5.7323	1.5126	
Z	N / A^a	10.4743	31.256	
16 Jul	50	3.1165	2.4011	
Z	9.8	17.3625	34.6806	
9 Aug	0	1.8597	1.4797	
Z	0.5657-	4.9866	7.8127	
16 Aug	1.1428	1.9495	1.2146	
Z	0.1428	12.65	5.1586	
30 Aug	1.0714	1.7418	1.3796	
z	0.0737	12.6587	3.9707	
13 Sep	2.3472	1.4184	2.438	
Z	13.3122	2.5098	6.4716	
23 Sep	14.4186	2.4504	2.1529	
z	37.4925	14.4606	8.1304	
30 Sep	4.9615	5.9853	2.0094	
Z	3.2016	28.2453	5.5953	
7 Oct	10.0855	1.9126	2.8975	
Z	19.6954	4.3067	7.8669	
15 Oct	6.8332	4.3967	2.7442	
Z	3.3472	27.5259	8.6776	
22 Oct	0	8.9632	1.1567	
Z	-2.5608	25.4903	14.6622	

^{*a*} at *N/A* indicates periods when *E. frosti* were not recovered in the samples

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Date	E. frosti	T. longisetosus	Z. mali	
9-Jul	N / A^a	-0.3	4.1746	
16-Jul	1.0197	0.3364	0.8053	
9-Aug	0.9690	0.9712	2.0601	
16-Aug	0.8461	0.6673	-0.3	
30-Aug	0.9608	1.9865	1.7018	
13-Sep	0.3688	0.9196	0.5955	
23-Sep	1.1871	1.1589	1.0236	
30-Sep	1.0168	1.1888	0.3146	
7-Oct	1.1388	0.266	-0.0422	
15-Oct	-0.1	0.2606	0.2092	
22-Oct	0.9989	-0.3	1.1567	

Table 3. k (coefficient of aggregation) values for three species at different dates .

^{*a*} at *N*/A indicates periods when *E. frosti* were not recovered in the samples.

alternation of sampling plan for an accurate population count (Slone and Croft, 1998b).

The results of population fluctuation revealed that T. longisetosus occurred earlier in the season than E. frosti. In addition, the highest densities of T. longisetosus occurred where E. frosti occurred at low densities. Early occurrence of T. longisetosus may increase predator densities that will prevent heavy infestation by E. frosti. Additionally the presence of T. longisetosus late in the season may sustain the predator's density. This would allow the survival of predators that will feed on the next year's populations of E. frosti. The density of Z. mali peaked on the average at 10.34 per leaf in early July, it had another increase in response to increasing density of T. longisetosus at mid August too. The density of E. frosti increased in mid September reaching a peak of 2.46 per leaf, just after a decrease in the mean density of Z. mali. The peak density of T. longisetosus occurred in late August reaching 4.4 per leaf. T. longisetosus population growth occurred after a decrease in the mean density of Z. mali too. Despite lower population density of host mites in early season, the population density of Z. mali was greatly higher than those of E. frosti and T. longisetosus because of the predator feeding on eggs and immature stages of other tetranychid and eriophyid mites. In addition it may suggest that Z. mali has other preys too to feed on them at early

season. An advantage of Z. mali is that it can survive for a long period of time in low prey densities (Villanueva and Harmsen, 1998). The ability of Z. mali to persist at low prey on alternative foods and its densities superior competitive abilities over phytoseiids may make it capable of contributing to significantly in biological control of pest mites (MacRae and Croft, 1996).

There are studies that have described the spatial distribution of tetranychid, tydeid and stigmaeid mites in apple orchards using dispersion indices that generally show aggregation or randomness behavior of these mites' populations. Aggregative spatial distribution of T. urticae in a carambola orchard has been reported by Shih and Wang (1996). Croft and Slone (1997) supported the general hypothesis that regulation of P. ulmi occurred at the lowest equilibrium levels in plots with the most diversity of predators. Greco et al. (1999) demonstrated that spatial distribution of T. urticae and its predator N. californicus on strawberry was aggregative and N. californicus exhibited lower aggregation than T. urticae. Duso et al. (2005) demonstrated that densities of T. caudatus increased in late summer when downey mildew symptoms occurred on a high number of leaves. The density of its predator (Paraseiulus talbii Athias-Henriot) increased in late season following the increase of tydeids. So (2006) suggested that spatial distribution of *T. urticae* was nonrandom and followed negative binomial distribution. Although some studies have used chi-square goodness of fit test to compare the observed and expected frequency data under the negative binomial or Poisson distributions, this statistical procedure is not usually recommended. Villanueva and Harmsen (1998) revealed that *Z. mali* was more abundant in the pyrethroid sprayed plots than in the control plots. Therefore *Z. mali* would be a good chance as a biological control agent in IPM programs through use of pyrethroids to reduce pest densities.

Spatial distribution pattern of the examined mites, using different analytical methods, was obtained aggregated or randomness, suggesting that the different statistical methods have various results and accuracy in calculating spatial distribution of an organism. The other deduction would be that the spatial distribution of studied mites was in the border of aggregated and random distributions. Iwao's α and β and Taylor's a and b values (Table 1) indicated a random distribution pattern for all species. Taylor (1961) and Taylor et al. (1978) interpreted coefficient *a* as a computing factor dependent upon sample size and method of variance estimation population and coefficient b as the true population statistic or index of aggregation with a continuous gradient from near random distribution (b=1) through regular (b<1) to aggregated r^2 values of two (*b*>1). Comparing analytical methods (Table 1) revealed that Taylor's power law gives more accurate estimation of populations than Iwao's patchiness regression.

Aggregated spatial distribution of all species was evaluated by index of dispersion (I_D) , Lloyd's mean crowding and Morisita's coefficient.

Slone and Croft (1998a) found that stigmaeid and phytoseiid predators were less aggregated than spider mites. *T. urticae*, *Eotetranychus* sp., *P. ulmi* and *Z. mali* were more clumped when their densities were low. Aggregation of *T. pyri* was related to aggregation and densities of *P. ulmi* and *Z. mali*. Comparing k values among three species showed that *Z. mali* was the least and *T. longisetosus* the most aggregated species in early July where their mean density were 10.34 and 0.37 per leaf, respectively. During the season with decreasing predator's density, the coefficient of aggregation increased too. These results confirm the results of Slone and Croft (1998a) studies.

The non-linear response of Z. mali to prey density is more difficult to explain. Lawson and Walde (1993) estimated that Z. mali which has been thought to be less important in control of P. ulmi, showed a stronger response than T. pyri to the presence of prey density. Because of its wide host range among other phytophagous mites, it is suggested that Z. *mali* acts density independent as the regression between predator and prey densities was not significant. Since Z. mali does not rely solely on prey for its nutrition but rather appears to eat leaf tissues as well, it could respond directly to leaf condition (Lawson and Walde, 1993). Clements and Harmsen (1992) used simulation model to examine the interactions between predatory mites of two families, Stigmaeidae and Phytoseiidae, representatives of which feed on phytophagous apple mites.

At low prey densities, stigmaeids held an advantage over phytoseiids in terms of efficacy because of their higher preference for prey eggs, higher oviposition relative to prey consumption and the ability to consume their own eggs, thus the weaker predator (Stigmaeidae) is the more effective competitor at low prey densities. A combination of stigmaeids and phytoseiids was shown to have greater efficacy than either predator alone over a wide range of prey densities (Clements and Harmsen, 1992).

The population of *Z. mali* is established early season on apple orchards as compared to its preys, therefore it can act as an effective predator in biological control programs based on conservation or augmentation in IPM strategies.

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فعالیت فصلی کنه شکار گر (Stigmaeidae) فعالیت فصلی کنه شکار گر (Tetranychid) Tydeus longisetosus (Tydeid), Eotetranychus frosti در باغات سیب سمپاشی نشده مراغه

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چکیدہ

در این تحقیق فعالیت فصلی و الگوی توزیع فضایی کنه های Zetzellia mali Ewing و longisetosus (ElBagouy and Momen) مراغه در سال ۸۵ مورد بررسی قرار گرفت و نوع رابطه بین شکار گر و طعمه های آن تعیین گردید. برای تعیین الگوی توزیع فضایی از روش های تیلور، آیواو، موریسیتا، شاخص ازدحام لوید و شاخص پراکندگی استفاده شد. الگوی توزیع فضایی از روش های تیلور، آیواو، موریسیتا، شاخص ازدحام لوید و شاخص پراکندگی استفاده شد. الگوی توزیع فضایی این کنه ها با استفاده از روشهای تیلور و آیواو از نوع تصادفی و با استفاده از سایر روشها از نوع تجمعی تعیین گردید. نتایج مربوط به فعالیت فصلی کنه ها نشان دادند که اوج تراکم کنه های . ورشها از نوع تجمعی تعیین گردید. نتایج مربوط به فعالیت فصلی کنه ها نشان دادند که اوج تراکم کنه های . ورد شکار گر آنها (۲۰/۳۲ کنه در هر برگ) در اوایل آن (به ترتیب ۲/۴۶ و ۲/۴ کنه در هر برگ) و در مورد شکار گر آنها (۲۰/۳۲ کنه در هر برگ) در اوایل تیر ماه اتفاق افتاد. نوع ارتباط بین شکار گر و طعمه های آن نیز از نوع مستقل از تراکم تشخیص داده شد.