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Research Article

Population fluctuation and spatial distribution of cynipid oak gall forming wasps (Hymenoptera: Cynipidae) in Kermanshah, Iran

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Abstract Oak trees form the major elements of the Zagros forests at western part of Iran. The activity of cynipid gall wasps (Hymenoptera: Cynipidae) greatly affects survival and fertility of these trees, by inducing formation of various galls on a certain part of the oak trees (*Quercus* spp.). In this study, population fluctuation and spatial distribution of four common species, *Andricus asetivalis* (Giraud), *Aphelonyx persica* (Melika), *Neuroterus lanuginosus* (Giraud) and *Neuroterus saliens* (Kollar) were studied in Kermanshah province from the early February to the late December of 2011. The galls that formed on the 100 cm end of branches from the four cardinal directions were sampled in two different heights of 1.5 and 2.5 m on each tree every 7-10 days throughout the season. To estimate the spatial distribution patterns, data were analysed through regression models. According to the Taylor's power model, in the height of 1.5 m, the slope of regression line was not significantly different from 1, indicating a random pattern for all gall wasp species. Also the spatial distribution pattern of *N. lanuginosus* and *N. saliens* was determined as random in the height of 2.5 m. Using Iwao's patchiness regression, similar patterns were distinguished for all species in two different heights except for *A. aestivalis* and *N. lanuginosus* in the height of 1.5 m which regression between Lloyd's mean crowding and mean density was not significant. At early season, just sexual galls of *A. aestivalis* and *A. persica* were observed and the first asexual galls of *N. lanuginosus* and *N. saliens* were formed on the branches starting in early August. Combining data of both heights of 1.5 and 2.5, the peaks of gall density of *A. aestivalis*, *A.* persica, *N. lanuginosus* and *N. saliens* were observed on 23 May, 27 June, 4 September and 15 September as 2.85 ± 1.60, 34.40 ± 22.17, 159.27 ± 40.01 and 4.50 ± 1.38, respectively. Overall, the most observed galls belonged to *N. lanuginosus.* The activity period of *N. saliens* was clearly shorter than other species and lasted only less than two months.

Keywords: *Quercus*, seasonal fluctuation, gall-maker, Cynipidae

Introduction

Gall, as a neglected form of insect herbivory, is a plant tissue induced by another organism to provide food and physical protection for gall-makers (Cornell, 1983; Askew, 1984; Price and Pschorn-Walcher, 1988; Stone *et al*., 2002). Gall induction has evolved many times in various insects orders, which is assumed to be a special relationship between the insect and its host plant (Bihari, 2011). More than 15, 000 gall-inducing insect species that are grouped in seven orders have been described (Rohfritsch and Shorthouse, 1982). Gall-forming insects oviposite in leaves or other

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tissues, and then cause cell tumours with increased nutrient content near developing larvae and increased levels of tannins and fibres in the outer layers of galls (Ribeiro and Basset, 2007).

Oaks are reported to be primary hosts for a larger number of plant pathogens and insect herbivores (Stone *et al*., 2002). Cynipid wasps as obligate parasites on oaks (Stone *et al*., 2002) and the second most diverse family after cecidomyiid midges, are restricted to one or few host species (Abrahamson *et al*., 1998, 2003). Oak gall wasps (Hymenoptera: Cynipidae: Cynipini), as the obligate parasites of the oak trees (Stone *et al*., 2002), comprise one of the most species-rich groups of the gall inducing insects, and are capable of making an extensive variety of structures on their host plants. Each species creates a very complicated gall (Stone and Cook, 1998; Bihari, 2011). Gall induction in Cynipidae begins in response to chemicals, secreted by growing wasp larvae. There are about 1300 species of cynipid oak gall making wasps worldwide, majority of them occurring in the Nearctic (Cornell, 1983; Ronquist and Liljeblad, 2001; Stone *et al*., 2002). Most of them have a parthenogenetic life cycle (Atkinson *et al*., 2002) with sexual generation and an asexual (agamic) generation that develop in the spring or the early summer and in the summer and the autumn of the same year, respectively (Stone *et al*., 2002).

Knowledge about population fluctuations of insects at particular times and places is of fundamental interest in ecology (Byers, 2012). An insect population always fluctuates according to the dynamic conditions of its environment. Both physical (abiotic) and biotic factors are believed to be the factors responsible for the changes in a population. Andrewartha and Birch (1954) distinguished four elements of the environment that could affect insect populations as follows: climatic condition, food, other insects or organisms causing diseases and its habitats. Climatic factors (as temperature, rainfall and relative humidity) have great impact on insect population change (Way and Heong, 1994; Zhu, 1999; Siswanto *et al*., 2008). Most of the time, the density of insects is fluctuating and the analysis of such fluctuations are considered as one of the major fields in entomological researches (e.g. Cappuccino and Price 1995; Huffaker *et al*., 1999). Spatial distribution is considered as one of the most important ecological characteristic of communities that is related to the preference of habitat by the organisms (Connell, 1983). Organisms interact mostly with the neighbouring individuals of their own or other species (Tilman *et al.*, 1997).

Spatial distribution is important because it has been used in ecology, population sampling and pest management strategies (Legendre and Fortin, 1989). Spatial dispersion of insects provides the estimation of pest densities which are the basis for making decision in pest management (Southwood, 1978; Taylor, 1984; Perry, 1997; Southwood and Henderson, 2000).

The galls of cynipid are the well-structured community with the oak tree as primary producer, the gall wasp (s) and inquilines as grazers, and parasitoid, predators and fungi as natural enemies (Stone *et al*., 2002). Therefor these galls have been considered as a model system in community ecology (Askew, 1975).

In this study, we investigated on population fluctuations of *A. aestivalis*, *A. persica*, *N. lanuginosus and N. saliens*, the major oak gall wasp species in Kermanshah, and their spatial distribution at two different heights on oak trees.

Materials and Methods

Study area and sampling procedure

This study was carried out in the Chalabeh region of Kermanshah province (1500-2200 m altitude, 34˚22′55″N and 47˚16′29″E) from early February through to late December 2011. The annual mean temperature in this area is 14-15 °C with an annual precipitation of 373-436 mm. The natural vegetation is characterized by deciduous broadleaf forests, especially oak trees. The oak trees, *Quercus brantii* Lindl., in Chalabeh region are surrounded by mountains and completely separated from the forests in other regions The sampling was performed according to a stratified random sampling pattern. At each sampling date fifteen oak trees were randomly selected and all galls formed on the 100 cm end of the branches from the four cardinal directions of the trees and at two different heights of 1.5 and 2.5 m (as two strata) were sampled. This procedure was repeated every 7 to 10 days throughout the season. All counts were performed *in situ*. Since this sampling procedure was very time-consuming, the fifteen oak trees was the maximum number we were able to study for the required data on each sampling date.

Spatial distribution

Two methods were used to determine regression analysis; Taylor's power law (Taylor, 1961) and Iwao's Patchiness regression (Iwao and Kuno, 1968). Taylor (1961) stated that there is an exponential relationship between mean ($\bar{x}$) and variance (S^2), which is defined as the following equation:

$$S^2 = a\bar{x}^b \qquad \text{(Eq. 1)}$$

Where S^2 is the variance; $\bar{x}$ is sampling mean; *a* is related to sampling and it is the function of the environment and sampling units selected and *b* is the index of species aggregation. We can use the log transformation for estimating the coefficients of linear regression using the following formula:

$$Log(S^2) = Log(a) + bLog(\bar{x}) \qquad \text{(Eq. 2)}$$

Where *a* and *b* are intercept and slope of linear regression, respectively (Taylor, 1961). When *b* is 1, < 1 and/or > 1, the spatial distribution will be random, regular and/or aggregated, respectively.

Iwao's patchiness regression method was also used to specify the relationship between index of mean crowding (m^*) and mean ($\bar{x}$) using the following equation:

$$m^* = \bar{x} + \frac{S^2}{\bar{x}} - 1 \qquad \text{(Eq. 3)}$$

$$m^* = \alpha + \beta\bar{x} \qquad \text{(Eq. 4)}$$

Where α shows the tendency to accumulation (positive) or dispersal (negative) and β reveals the distribution of population in space and is interpreted same as *b* of Taylor's power law (Iwao and Kuno, 1968). A statistical *t*-test was run to compare the calculated values of *b* or β with 1 using the following equation:

$$t_c = \frac{(b-1)}{SE_b} \qquad \text{(Eq. 5)}$$

Where SE_b is the standard error of *b* or β. The calculated values for *t* (t_c) compared with *t*-values of table ($df = n-2$; $\alpha = 0.05$). If $t_c < t$-table, the null hypothesis ($b = 1$) is accepted and the spatial distribution determined as random. If $t_c > t$-table, the null hypothesis is rejected while $b > 1$ or $b < 1$ show that the spatial distribution is aggregated or regular, respectively.

Results

Population fluctuation

The population fluctuation of different gall wasps species are shown in Figs. 1 and 2. The galls of *A. aestivalis* were first observed on both height of 1.5 and 2.5 m on 23 May and then, several peaks were detected until 26 October. The greatest population peak of *A. aestivalis* at the height of 1.5 m occurred on 9 August; while, no distinct peak was observed at the height of 2.5 m (Fig. 1. A). Relatively similar trend was observed for *A. persica* except that, clear peaks occurred simultaneously on 27 June in both heights of 1.5 and 2.5 m. On this date, a mean of 22.3 ± 16.3 and 12.1 ± 6.2 galls was counted per each sample unit on the mentioned heights, respectively (Fig. 2. A). The first galls induced by *N. lanuginosus* at both heights were observed on 18 July and a peak on 4 September. Unlike the *A. aestivalis* and *A. persica*, on the peak point, the number of galls formed at 2.5 m was greatly more than 1.5 m, so that on average, 59.9 ± 17.4 and 99.3 ± 23.9 new galls were counted on the heights of 1.5 and 2.5 m, respectively (Fig. 2. B). The first galls of *N. saliens* appeared later than the previous species and peaked on 15 September and 13 October on the height of 1.5 and 2.5 m, respectively. Combining the whole data of both heights, the population of *N. saliens* was peaked as 4.5 ± 1.4 galls on 15 September. In comparison to the other three species, duration of activity of *N. saliens* was remarkably shorter and the number of galls was less (Fig. 1. B).

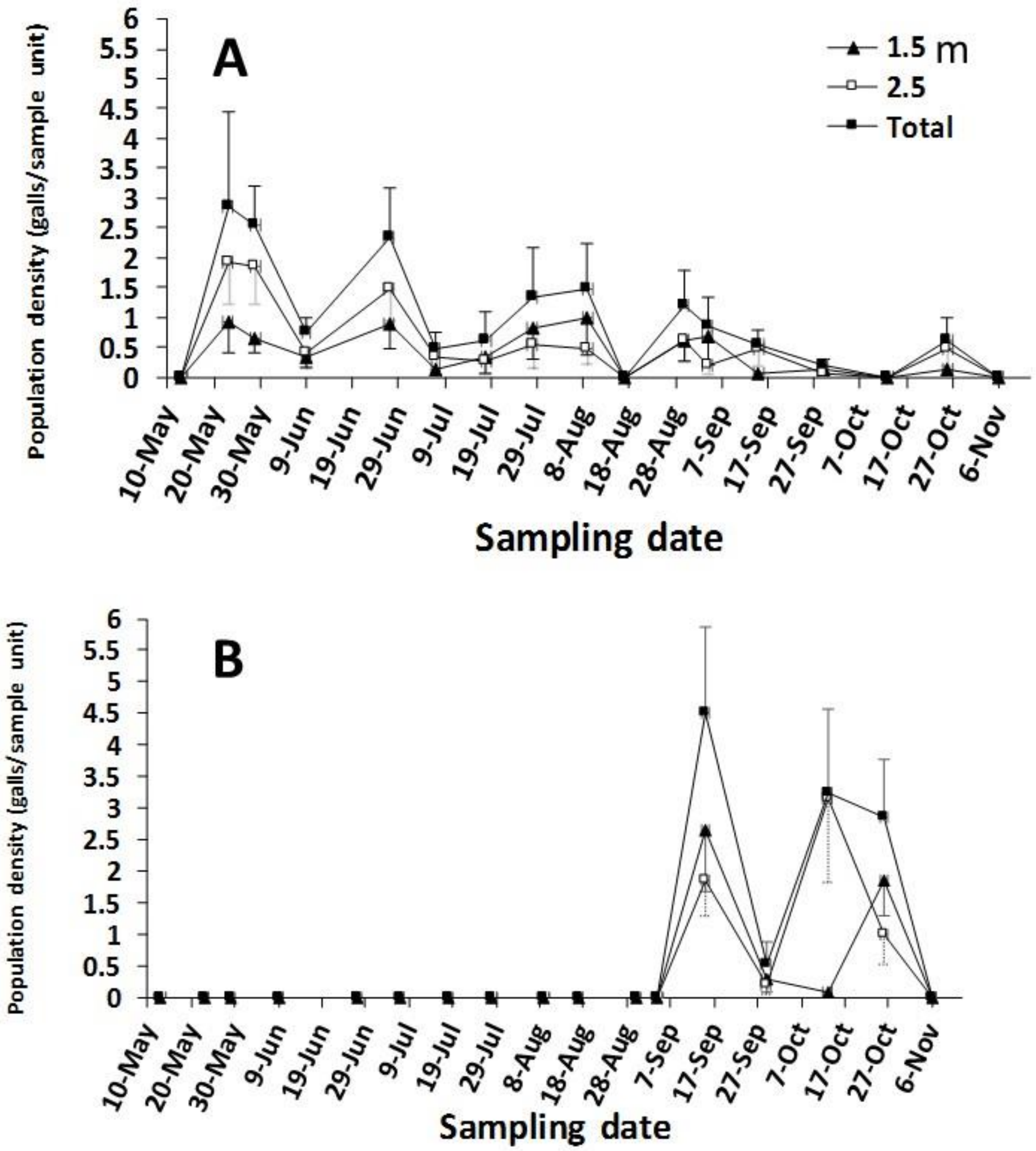
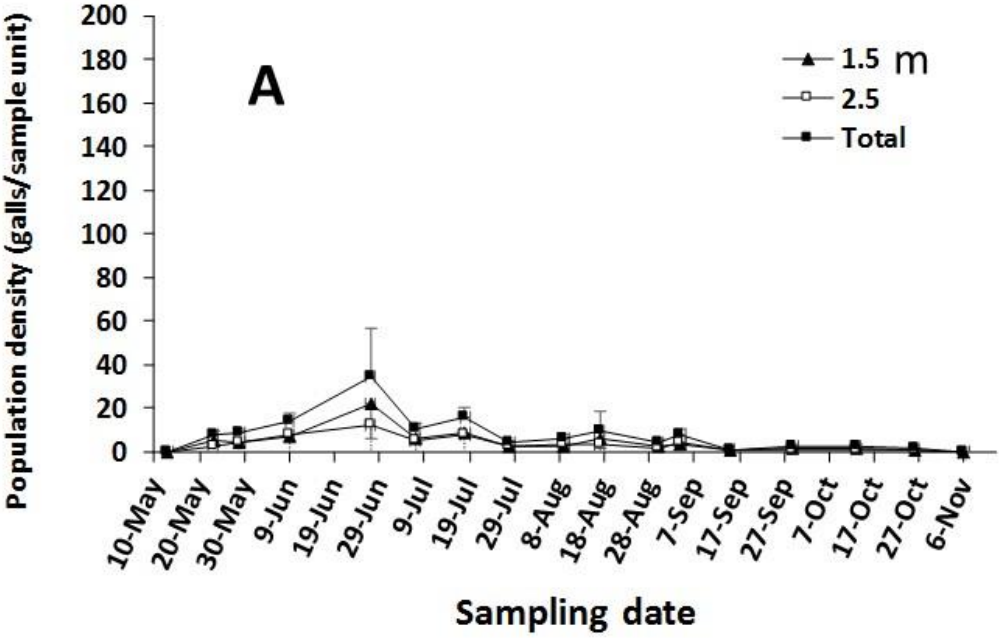


Figure 1 Population fluctuations of *Andricus aestivalis* (A) and *Neuroterus saliens* (B) during year 2011 in Chalabeh region, Kermanshah province.



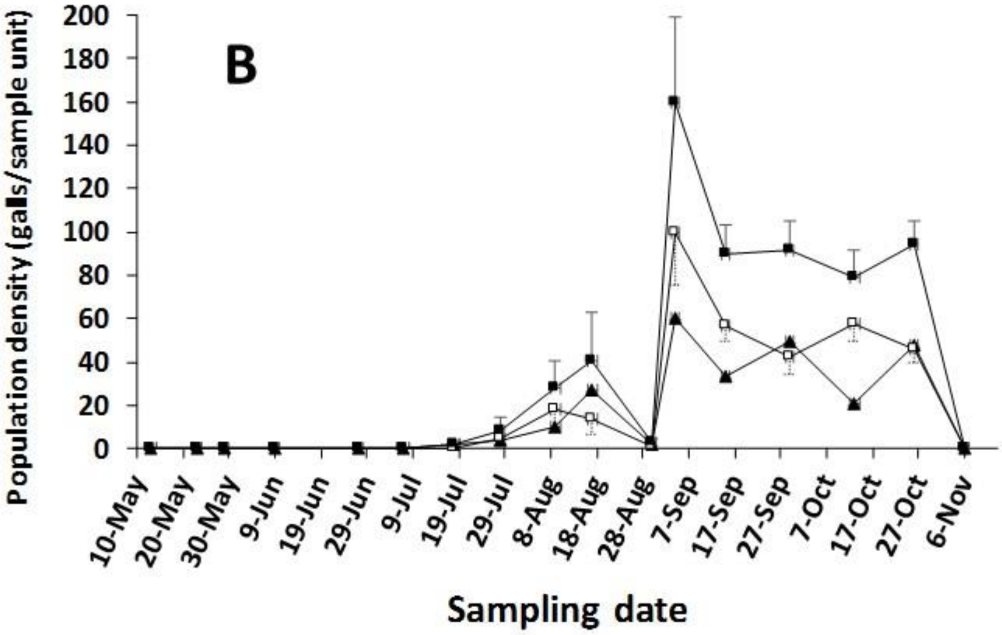


Figure 2 Population fluctuations of *Aphelonyx persica* (A) and *Neuroterus lanuginosus* (B) during year 2011 in Chalabeh region, Kermanshah province.

Spatial distribution

The results of Taylor's power law and Iwao's patchiness regression models in determination of spatial distribution pattern of the four cynipid gall wasps are depicted in Table 1. Regarding the obtained results by Taylor's power law model, a significant relationship was detected between $LogS^2$ and $Log\bar{x}$ for both heights of 1.5 and 2.5m (Table 1; $P_{value} \leq 0.05$). For the height of 1.5m, the slope of regression line was not significantly different with 1 indicating a random pattern for all gall wasp species. Also a random spatial pattern was recognized for *N. lanuginosus* and *N. saliens*at the height of 2.5 m, while the *b*-value of Taylor's model for *A. aestivalis* and *A. persica* at the height of 2.5m was noticeably greater than 1 indicating aggregated distribution. Based on the results of Iwao's patchiness regression, the relationship between mean crowding (m^*) and mean ($\bar{x}$) was not statistically meaningful (Table 1; $P_{value} > 0.05$) at the height of 1.5m for *A. aestivalis* and *N. lanuginosus;* therefore in this condition, the spatial distribution of these species could not be determined through Iwao’s method. In other cases, the slope of regression line was not significantly different with 1 and indicated a random spatial distribution, except for *A. persica* at the height of 2.5m at which the slope was greatly different from 1. Consequently, like the Taylor’s model, an aggregated spatial distribution was distinguished for *A. persica* at the height of 2.5m.

Table 1 Estimated parameters of Taylor's power law and Iwao's patchiness regression models for determination of spatial distribution patterns of the four cynipid gall wasp species.

Species	Index	Height (m)	b (± SE) (CI 95%)[1]	a (± SE) (CI 95%)	Tc[2]	R^2	P-value
Andricus aestivalis	Taylor	1.5	1.09 ± 0.32 (0.39-1.79)	0.33 ± 0.20 (-0.12-0.77)	0.29	0.52	0
		2.5	1.32 ± 0.09 (1.11-1.53)	0.50 ± 0.05 (0.39-0.62)	3.49*	0.95	0
	Iwao	1.5	2.54 ± 1.33 (-0.40-5.47)	0.98 ± 0.86 (-0.91-2.87)	1.15	0.25	0.08
		2.5	1.90 ± 0.42 (0.97-2.82)	1.025 ± 0.33 (0.29-1.76)	2.13	0.65	0
Aphelonyx persica	Taylor	1.5	1.25 ± 0.20 (0.73-1.77)	0.29 ± 0.17 (-0.14-0.73)	1.25	0.89	0
		2.5	1.78 ± 0.14 (1.19-2.37)	0.81 ± 0.08 (0.45-1.16)	5.69*	0.99	0
	Iwao	1.5	2.16 ± 0.80 (0.100-4.22)	-0.13 ± 0.41 (-0.92-1.18)	1.45	0.59	0.04
		2.5	5.98 ± 0.56 (4.55-7.43)	-0.03 ± 0.20 (- 0.55-0.49)	8.81*	0.96	0
Neuroterus lanuginosus	Taylor	1.5	1.21 ± 0.25 (0.62-1.8)	1.22 ± 0.34 (0.421-2.01)	0.83	0.88	0
		2.5	0.99 ± 0.28 (0.83-1.74)	1.28 ± 0.19 (0.32-1.66)	0.04	0.93	0
	Iwao	1.5	1.33 ± 0.71 (-0.34-3.00)	31.64 ± 24.52 (-26.35-89.64)	0.46	0.33	0.10
		2.5	1.21 ± 0.36 (0.35-2.06)	25.87 ± 17.65 (15.86-67.06)	0.57	0.78	0.01
Neuroterus saliens	Taylor	1.5	1.40 ± 0.10 (0.98-1.82)	0.46 ± 0.06 (0.18-0.73)	4.08	0.99	0
		2.5	1.46 ± 0.19 (0.62-2.30)	0.46 ± 0.09 (0.11-0.86)	2.36	0.96	0.02
	Iwao	1.5	2.36 ± 0.40 (-0.64-4.09)	0.09 ± 0.65 (-2.71-2.89)	3.4	0.95	0.03
		2.5	2.77 ± 0.61 (0.15-5.38)	0.10 ± 1.16 (-5.11-4.90)	2.91	0.91	0.04

[1] 95% confidence interval.
[2] The calculated *t* for slope (b). a indicates intercept.
* Different with $b = 1$ in significant level of 0.05.

Discussion

While, some studies have been carried out regarding cynipid gall wasps fauna of oak forests in the western parts of Iran (Azizkhani et al., 2007; Hosseinzadeh, 2011; Nazemi *et al*., 2008;), in the current study for the first time, population fluctuations and spatial distribution of the four cynipid species were investigated according to a regular sampling program.

In fundamental ecology, the interaction between host plants and gall-forming insects is considered as one of the most interesting topics by the expert ecologists. Hereof, various aspects of this relationship such as evolution and structure of induced galls (Abrahamson *et al*., 1998; Ronquist and Liljeblad, 2001; Stone and Cook, 1998); mechanism of gall formation (Ribeiro and Basset, 2007); the secondary metabolites of galls (Cornell, 1983; Abrahamson *et al*., 2003); biology and behavior of gall-maker insects (Askew, 1984; Atkinson *et al*., 2002; Stone et al., 2002) and gall-maker insects-parasitoid interaction (Price and Pschorn-Walcher, 1988) have been investigated.

Nevertheless, we found no references about spatial distribution pattern of cynipid gall wasps and our findings are unique in this regard. The spatial distribution of the leaf galls induced by *Callirhytis cornigera* (Osten Sacken) is assessed by Eliason and Potter (2001) within leaves, shoots, canopy and whole tree level and they found that within leaves, proportionately more galls were located on primary lateral veins as compared with the midveins, petioles and secondary lateral veins and the most shoots had < 20 galls, however, some current-year shoots c ontained > 100 leaf galls. They also reported that the leaf gall density was not significantly different among heights within individual tree canopies, but was highly variable between trees.

In a similar research, Giertych *et al*. (2013) investigated on spatial distribution of *Cynips quercusfolii* L. galls on leaves and within crown of oak trees. They concluded that regardless of the leaves size, the galls were located at a fixed distance from the edge of leaves; while, the distance to the leaf petiole depended significantly on leaf size. They also found that the number of galls did not depend on tree height but depended on the position of the leaf within the crown. Both studies fundamentally diffe from our research. Since, both Elison and Potter (2001) and Giertych *et al*. (2013) studied on spatial distribution pattern of galls within each individual oak tree and described how the gall makers shared their eggs at a level of leaf, shoot or whole tree. In contrast with earlier research, in the current study, the spatial distribution of induced galls by the four cynipid species has been studied at the landscape level.

The obtained results revealed how cynipid wasps distribute their galls among available oak trees in a given landscape. Meanwhile, Elison and Potter (2001) and Giertych *et al.* (2013) determined spatial distribution pattern through comparison of the mean number of induced galls at various parts of an individual tree. While, we obtained the spatial distribution pattern according to the two well-known statistical procedures (Taylor’s power law and Iwao’s patchiness regression) which are approved by the ecologists (Perry, 1997; Krebs, 1998; Southwood, 1978; Southwood and Henderson, 2000).

According to the obtained results by Taylor’s power law and Iwao’s patchiness regression methods, we found that the galls induced by *N. lanuginosus* and *N. saliens* were distributed randomly in the studied area at both heights of 1.5 and 2.5m above ground level. However, the spatial distribution of galls formed by *A. aestivalis* and *A. persica* varied between the two heights; random at height of 1.5m and aggregated at 2.5m. It is noteworthy that different patterns of aggregated and random was obtained for galls of *A. aestivalis* at the height of 2.5m using Taylor and Iwao’s methods, respectively. Hereof, based on the R^2-values, the Taylor method was selected which showed a better fitness with experimental data. The random distribution showed that the presence of an individual on a tree is not affected by the presence of another, and that all trees have a similar chance of being occupied by an individual (Southwood, 1978).

A large overlap was observed between gall induction periods of *A. aestivalis* and *A. persica* in the studied area, both species started on 23 May and ended on 26 October. On the other hand, the first galls of *N. lanuginosus* and *N. saliens* appeared about two and four months later, respectively. Surprisingly however, the gall induction by all the species ended exactly on the same date, 26 October. These findings proved that *N. saliens* and *N. lanuginosus* are more thermophilic than *A. aestivalis* and *A. persica,* yet all species require the same minimum temperature to continue their activities.

The density of galls induced by the *A. aestivalis* and *A. persica* at the height of 1.5m was more than 2.5m, but a different situation was observed about galls of *N. lanuginosus* and *N. saliens.* These findings revealed that the two latter species have a different behavior in occupation of the favorable height of a tree and prefer higher canopies than *A. aestivalis* and *A. persica.* Among the four studied cynipid species, the maximum number of galls was induced by *N. lanuginosus* and galls of *N. saliens* were rarely observed.

Our findings can be useful to understanding a small part of the complex relationship between oak trees and cynipid gall wasps. Certainly, much more efforts should be made to increase our knowledge about various behavioral aspects of cynipid wasps.

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نوسانات جمعیت و توزیع فضایی زنبورهای گال‌زای بلوط (Hymenoptera: Cynipidae) در کرمانشاه

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چکیده: درختان بلوط به‌عنوان مهم‌ترین جزء از ساختار گیاهی جنگل‌های زاگرس غرب ایران محسوب می‌شوند. زنبورهای گال‌زای خانواده Cynipidae با القای تشکیل گال‌های متعدد روی درختان بلوط، *Quercus* spp.، تأثیر قابل‌توجهی روی بقا و زادآوری آنها می‌گذارند. در این تحقیق، نوسانات جمعیت و توزیع فضایی چهار گونه مهم از زنبورهای گال‌زا شامل *Andricus aestivalis* (Giraud)، *Aphelonyx persica* (Melika)، *Neuroterus lanuginosus* (Giraud) و *Neuroterus saliens* (Kollar) از اواسط زمستان سال ۱۳۸۹ تا اوایل زمستان سال ۱۳۹۰ در استان کرمانشاه مطالعه شد. برای این منظور، تعداد گال‌های هر یک از گونه‌های مورد مطالعه روی ۱۰۰ سانتی‌متر انتهایی شاخه‌های اصلی، در چهار جهت جغرافیایی مختلف و در دو ارتفاع ۱/۵ و ۲/۵ متر از سطح زمین در هر هفت الی ده روز شمارش و ثبت شد. برای تعیین نوع الگوی توزیع فضایی، از مدل‌های رگرسیونی استفاده شد. براساس قانون توان تیلور، در ارتفاع ۱/۵ متر از سطح زمین، شیب خط رگرسیون تفاوت معنی‌داری با عدد یک نداشت و بر این اساس توزیع فضایی هر چهار گونه مورد مطالعه از نوع تصادفی تعیین شد. در ارتفاع ۲/۵ متر از سطح زمین نیز برای گونه‌های *N. lanuginosus* و *N. saliens* توزیع فضایی تصادفی به‌دست آمد. با استفاده از مدل رگرسیونی آیوائو نیز الگوی توزیع فضایی مشابهی برای گونه‌های مورد مطالعه به‌دست آمد، اگرچه در مورد گونه‌های *A. aestivalis* و *N. lanuginosus* در ارتفاع ۱/۵ متر، رگرسیون بین میانگین تراکم جمعیت و میانگین ازدحام به لحاظ آماری معنی‌دار نبود. گال‌های جنسی گونه‌های *A. aestivalis* و *A. persica* از ابتدای فصل روی شاخه‌ها تشکیل شدند، ولی گال‌های غیرجنسی گونه‌های *N. lanuginosus* و *N. saliens* از اواسط تابستان به بعد مشاهده شدند. در تجمیع داده‌های هر دو ارتفاع، زمان اوج تشکیل گال‌های گونه‌های *A. aestivalis*، *A. persica*، *N. lanuginosus* و *N. saliens* به‌ترتیب در روزهای سوم خرداد، چهارم تیر، ششم شهریور و پانزدهم شهریور و برابر با ۱/۶۰ ± ۲/۸۵، ۲۲/۱۷ ± ۳۴/۴۰، ۴۰/۰۱ ± ۱۵۹/۲۷ و ۱/۳۸ ± ۴/۵۰ گال ثبت گردید. در مجموع، بیش‌ترین تعداد گال مشاهده شده روی درختان به گونه *N. lanuginosus* تعلق داشت و دوره فعالیت زنبور *N. saliens* به‌طور مشخصی کوتاه‌تر از سایر گونه‌ها بود و کم‌تر از دو ماه به‌طول انجامید.

واژگان کلیدی: بلوط، نوسانات فصلی جمعیت، زنبورهای گال‌زا، Cynipidae