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Bioecology of the pear lace bug, *Stephanitis pyri* (F.) (Hemiptera: Tingidae) on walnut trees in Kermanshah Province, Iran

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Abstract. One of the most serious pests of walnut trees in recent years in Kermanshah Province is the pear lace bug, *Stephanitis pyri* (F.) (Hemiptera: Tingidae). Damage of lace bug is not usually economic, but serious invasions can cause considerable damage or even death to the host. A field study was carried out to evaluate the fluctuations of *S. pyri* population on walnut trees in Kermanshah from January to December 2015. Data were analyzed using Taylor's power law and Iwao's patchiness regression methods to determine spatial distribution. In order to estimate the biological parameters of *S. pyri* in two different laboratory and semi-field conditions, the theory of age-stage two-sex life table was used. None of the life table parameters (r: intrinsic rate of natural increase; R_0: net reproductive rate; λ: finite rate of increase; T: mean generation time; and, GRR: gross reproductive rate) in the laboratory conditions had a significant difference from the field conditions, and more than 70% of individuals successfully passed the pre-imaginal developmental stages. The pear lace bug overwintered as adult within weeds until late March; after that, they migrated to the walnut trees and distributed on the leaves. As a result, there was no significant relationship between the fluctuations of the pear lace bug population and changes in temperature or relative humidity. Spatial distribution of the pear lace bug population was determined based on both Taylor and Iwao regression methods of aggregated type.

Keywords: *Stephanitis pyri*, life table, population fluctuation, spatial distribution

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Introduction

The walnut tree, *Juglans regia* L., is one of the oldest fruit trees that has been used by humans since 7000 years ago (Clark *et al.*, 2007) and has various efficiencies in agriculture, forestry, pharmaceuticals, *etc.* (Aradhya *et al.*, 2006). Different factors, such as pests and diseases, can affect the quality of walnuts. Recently, one of the most serious pests of walnut trees is the pear lace bug, *Stephanitis pyri* (F.) (Hemiptera: Tingidae). *S. pyri* has been successfully established on Rosaceae, especially Juglandaceae plants in Mediterranean regions (Aysal & Kivan, 2008). Damage of *S. pyri* is not usually economic, but in serious invasions, significant damage or even death of host plants can happen (Froeschner, 1995). The *S. pyri* adults and nymphs feed on the leave's underside by sucking plant sap. Pear lace bugs insert their stylet through the lower epidermis of leaves and consume nearly all of the parenchyma cells under the upper epidermis, resulting in chlorosis of leaves (Buntin *et al.*, 1996). Feeding of nymphs and adults leads to the formation of small black or dark brown spots on the upper surface of the leaves. On the underside of the leaf, the insect generates shiny black spots related to its excreta; infected leaves are fed with chlorophyll. Affected trees will be weakened and lose leaves before autumn. Besides, the fruits of infected trees shrink and drop, resulting in decreased yield in the same or the following year (Vergnani & Caruso, 2008).

The pear lace bug is a small and broad insect; the body length of the insect is about three to four millimeters. It is grayish-brown and tiger-shaped; the front and - hind wings are lace-shaped and translucent, and the length of the

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wing is almost twice the length of the body. The oval-shaped nymphs of *S. pyri* are white and black, and their eggs are black and oval (Stonedahl *et al.,* 2008). As Neal and Schaefer (2000) explained, the eggs are deposited inside the spongy mesophyll of the leaves, next to the veins, and are entirely covered with varnish material. The pear lace bug is distributed from Europe, Russia, North Africa, and Eastern Mediterranean to Armenia, Iraq, and Iran (Schaefer & Panizzi, 2000).

For more efficient management, knowledge about the population growth parameters of pear lace bug is needed. Estimating population growth parameters and determining the type of insect population growth based on reproductive capacity is an indispensable study of insect population, which can be obtained using the life table. This study was carried out to determine the population fluctuations of the pear lace bug on walnut trees in Kermanshah province and to study the life table parameters in laboratory and field conditions. Therefore, in addition to assessing the spatial distribution of the bug, the relationship between population fluctuations of *S. pyri* and temperature changes along with the relative humidity of the day was also investigated.

Materials and methods

Population fluctuations

The field assessments were conducted from January to December 2015 on walnut trees in the Campus of Agricultural and Natural Resources of Razi University, Kermanshah Province, Iran. Samplings were randomly carried out from the walnut trees twice a week. The sampling unit consisted of five leaves at the end of the main branches. Sampling was conducted in four main directions and at different heights in each tree. Then, the number of various life stages of the lace bugs was counted *in situ* and recorded separately. A primary sampling was done with 30 sample units to determine the optimal number of samples. After that, the relative variation (RV) of the samples was calculated using the following equation:

$$\mathrm{RV} = \frac{\mathrm{SE}}{\bar{\mathrm{x}}}$$

where $\bar{x}$ is the average of primary sampling data, and *SE* is the standard error. This study considered the maximum relative error of 25% as acceptable. According to the relative error of the data obtained from the primary sampling, the following equation was used to determine the number of required samples (Southwood & Henderson, 2000):

$$\mathrm{N} = \left(\frac{t \times \mathrm{SD}}{\mathrm{D} \times \bar{\mathrm{x}}}\right)^2$$

where *N* is the number of required samples, *t* is the t-student table according to the degree of freedom of the sample number, *SD* is the standard deviation of the primary sampling data, and *D* is the maximum acceptable error (0.25). For the next samplings, according to the uniformity of the area and three to four days intervals between the samplings, the previous samplings data were used to determine the number of samples required for the subsequent samplings.

Spatial distribution

To determine the spatial distribution, the obtained sampling data were analyzed using Taylor's power law ($\log S^2 = \log a + b\ log\bar{x}$) and Iwao's patchiness regression method ($m^* = \alpha + \beta\bar{x}$) (Taylor, 1961; Iwao, 1968). where $\bar{x}$ is the mean density, S^2 is variance, *log a* and α are the intercept, *b* and β are the slope of the regression line, and m^* is Lloyd's mean crowding index ($m^* = \bar{x} + \frac{S^2}{\bar{x}} - 1$). Here, if *b* or β is 1, <1, or >1, then the spatial distribution will be random, regular, or aggregated, respectively.

Life table in the laboratory

The experiments were carried out inside Petri dishes, on the detached leaves of walnut, and in a growth chamber with 25±1°C, 65 ± 5% relative humidity, and a photoperiod of 16L: 8D hours. In this regard, a wet cotton was placed on the bottom of a Petri dish (8 cm diameter), and a piece of walnut leaf was placed with the lower leaf surface upward on it. After that, 1st instar nymphs were transferred separately to the leaf, and placed in a Petri dish. The walnut leaves were replaced with fresh ones every 3-4 days. All Petri dishes were checked every 24 hours.

Life table in the field

This part of the study was conducted on walnut trees and in natural environmental conditions of the pear lace bug's life in the spring and summer of 2015. The insect's breeding environment on the walnut trees was formed such that the end leaves of the walnut branches were enclosed into 30×30 cm sleeves, and the insects were grown individually on separate branches and inspected daily. This experiment started at the beginning of June and continued until the last week of August. The average daily temperature during this period varied between 22.4 and 30.1°C, and the average daily temperature for the whole period was 27.3°C.

Life table studies were conducted in laboratory and field conditions with 80 and 75 replications, respectively.

Data analysis

Life history data were analyzed according to the theory of age-stage, two-sex life table (Chi & Liu, 1985; Chi, 1988). To facilitate analysis of life table data, we used TWOSEX-MS-Chart program (Chi, 2013). The age-stage specific survival rate (S_{xj}; where x is age and j is stage), the age-specific survival rate (l_x), the age-specific fecundity (m_x), and the population parameters (r: the intrinsic rate of increase; λ: the finite rate of increase, R_0: the net reproductive rate; *GRR*: The gross reproductive rate, and T: the mean generation time) were calculated accordingly.

Individuals of the same age at different stages may have different life expectancies. Based on the age-stage, two-sex life table, we can calculate life expectancy for each individual (Chi, 2013). The parameters of pear lace bug life table were calculated according to the bootstrap method, and their statistical comparison was performed based on the reliability interval of mean differences (CI) (Smucker *et al.*, 2007) and done by Paired bootstrap test using the TWOSEX-MSChart (2016) at a 95% confidence interval. Linear regression was used to investigate the relationship between population fluctuations and daily mean temperature and relative humidity.

Results

Development

The duration of growth and development of each stage of *S. pyri* is presented in Table 1 for laboratory and field conditions. The results showed that there is no difference in the developmental periods of lace bug between laboratory and field conditions in different stages except for the first instar nymph and adult pre-oviposition period (APOP). Statistical comparison of data based on the Paired Bootstrap Test (PBT Pooled) shows that only the developmental time of the first instar nymph and APOP were different between the two mentioned conditions.

Table 1. Means (±SE) of developmental periods (days) of different stages of *Stephanitis pyri* in laboratory and field conditions

Life stage	Laboratory	Field
Egg	16.74±0.11a*	16.72±0.08a
Nymph 1	2.88±0.06a	2.66±0.23b
Nymph 2	2.71±0.06a	2.72±0.21a
Nymph 3	3.37±0.48a	2.52±0.25a
Nymph 4	2.75±0.06a	2.94±0.22a
Nymph 5	3.41±0.07a	3.31±0.25a
Female	57.51±1.85a	61.23±2.65a
Male	55.67±4.25a	60.25±4.33a
Total	47.71±2.23a	52.23±2.65a
Adult pre-oviposition period (APOP)	2.12±0.17b	3.28±0.19a
Total pre-oviposition period (TPOP)	12.56±0.88a	14.28±1.15a

* Significant difference (α=0.05; Paired bootstrap test with 100000 resamples)

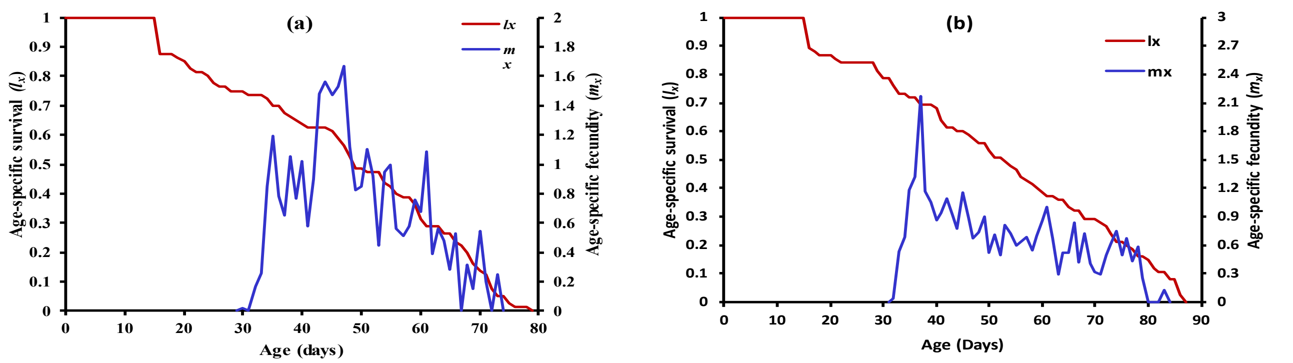


Fig. 1. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *Stephanitis pyri* in laboratory (a) and field (b) conditions

Life table parameters

Life table parameters of pear lace bug obtained under the laboratory and desert conditions are listed in more detail in Figs. 1-4 and Table 2. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *S. pyri* in laboratory and field conditions are shown in Fig. 1. The survival rate represents the ratio of the number of individuals surviving to age x and age-specific fecundity indicates the number of individuals produced at each age x per day. The death of the last individuals in the laboratory and field conditions was observed on the 79th and 87th days, respectively. In both experimental conditions, the mortality rates in immature stages was low, and more than seventy percent of individuals successfully grew to maturity. Fifty percent of mortality cohorts in laboratory and field conditions was observed on the50th and 53rd days, respectively. The first oviposition by females in both laboratory and field conditions was observed on day 31. The peak of oviposition in laboratory and field conditions was on days 48 and 38, and the average of 1.67 and 2.17 eggs per female was calculated, respectively.

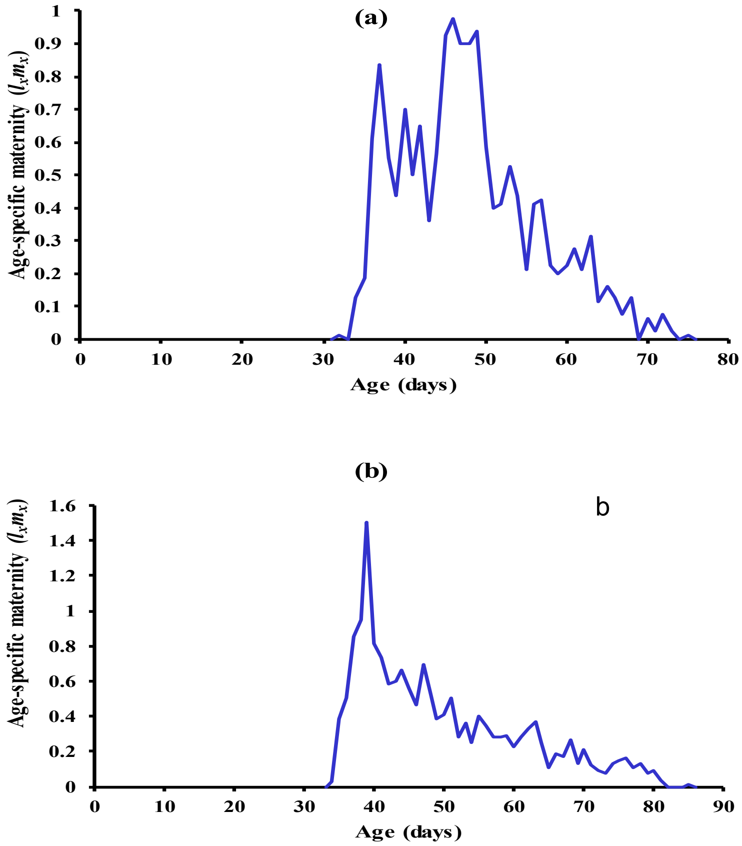


Fig. 2. Age-specific maternity ($l_x m_x$) of *Stephanitis pyri* in laboratory (a) and field (b) conditions

Table 2. Means (±SE) of life table parameters of *Stephanitis pyri* in the laboratory and field conditions

Parameter	Laboratory	Field
Intrinsic rate of increase (r) (d^{-1})	0.060±0.002a*	0.062±0.002a
Net reproductive rate (R_0) (offspring)	15.841±1.990a	17.412±2.251a
Finite rate of increase (λ) (d^{-1})	1.061±0.002a	1.063±0.003a
Mean generation time (T) (d)	45.022±0.522a	45.194±0.672a
Gross reproductive rate (GRR) (offspring)	32.043±2.701a	34.812±3.832a

*Means in the same row followed by the same letter are not significantly different (P>0.05) using paired bootstrap test with 100000 resamples

Age-specific maternity ($l_x m_x$), shown in Fig. 2, represents the number of individuals added to the population daily. In laboratory conditions, the peak of the $l_x m_x$ curve occurred on day 45 and was slightly different from the peak of the m_x curve. In field conditions, the peaks of the $l_x m_x$ and m_x curves were the same. Therefore, the population growth center was obtained on days 45 and 38 in laboratory and field conditions, respectively. The age-stage survival rate (S_{xj}) gives the probability that a newborn egg will survive to age x while in stage j (Fig. 3).

The first adult male and female insects appeared on days 31 and 32 in the laboratory and on day 31 in the field conditions, respectively. In each experimental condition, the longevity of male and female was almost equal. In both experimental conditions, the proportion of females was higher than males, and the sex ratio of the insect population was based on females.

Life expectancy indicates the number of remaining days of life of living individuals for each age *x* and stage *j*. For example, as shown in Fig. 4, on the 25th day of the experiment, the average remaining days in the life of the fourth instar nymphs was 30 days in laboratory conditions and 34 days in field conditions. In both laboratory and field conditions, the life expectancy of males was higher than that of female adults for a long duration of the *S. pyri* life span.

The results of estimating life table parameters and their statistical comparison are presented in detail in Table 2. The most critical parameter of the life table is the intrinsic rate of increase (*r*), which indicates the rate of population growth per day and the difference between the mortality rate and fertility rate. The values of this parameter for the pear lace bug in both experimental conditions were very close and lacked statistical significance. According to results of the Paired Bootstrap Test (PBT pooled), none of the life table parameters in the laboratory conditions had a significant difference from the field conditions.

The sex ratio of pear lace bugs in the laboratory was 3.91:1 (female: male), whereas the ratio was 1.95:1 (female: male) in the field conditions. The obtained ratios were compared with the expected 1: 1 ratio, and it was found that in both laboratory (χ^2=20.76; P_{value}<0.01) and field conditions (χ^2=6.12; P_{value}=0.01), the obtained sex ratio was significantly different from the expected ratio.

Population fluctuation

Sampling was performed during the entire growth season of 2015 (Fig. 5). Since late March 2015, the overwintering adults migrated from weeds to walnut trees and began their activity. The first egg-laying was on May 8, and the first age nymphs were seen from late May.

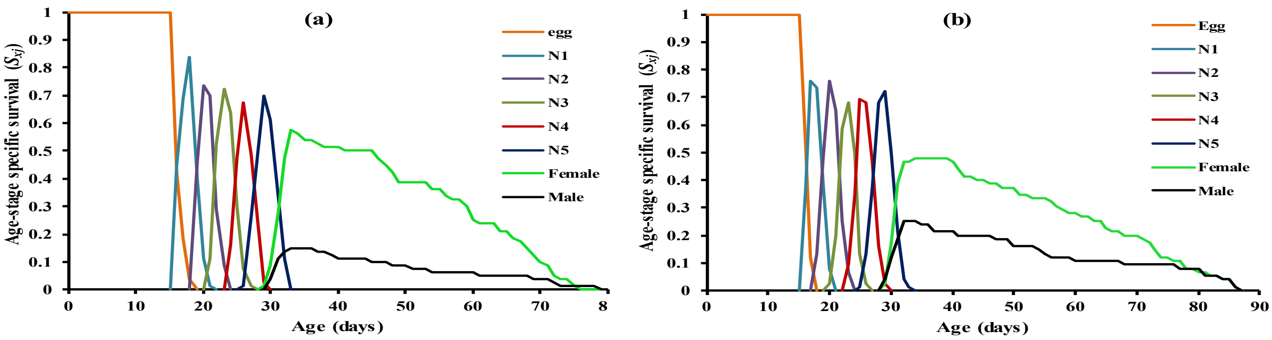


Fig. 3. Age-stage survival rate (S_{xj}) of *Stephanitis pyri* in laboratory (a) and field (b) conditions

Table 3. The results of spatial distribution of *Stephanitis pyri* population using Taylor's power law method

Life stage	Slope±SE	Intercept±SE	R^2	t_b	*f*	P_{value}
Nymph 1	1.127±0.104	0.710±0.147	0.951	1.221	117.6	<0.001
Nymph 2	1.300±0.166	0.842±0.234	0.885	1.807	61.4	<0.001
Nymph 3	1.214±0.118	0.701±0.163	0.930	1.814	105.9	<0.001
Nymph 4	1.308±0.091	0.818±0.135	0.953	3.385	205.0	<0.001
Nymph 5	1.298±0.059	0.832±0.083	0.982	5.051	488.1	<0.001
Adult	1.144±0.121	0.241±0.071	0.909	1.190	90.0	<0.001

The first peak of nymphs was recorded on June 2, and the first peak of the adult population on June 24, which totaled 0.63 ± 0.08 and 0.052 ± 0.05, respectively. The average population on August 16 reached 4.05 ± 0.48 per unit. It remained at its peak until October 11, then, with the migration of adult insects to the weeds for overwintering, it started its declining process and reached zero on the last day of November. The highest peak of nymphs, adult insects, and the total population were recorded on 2nd, 20th, and 2nd of September, respectively, with mean values of 5.83 ± 0.95, 6.04 ± 0.95, and 8.70 ± 1.23 in each sampling unit. According to the population fluctuation graphs, the insect has three generations per year, and the overlap between the generations is quite evident (Fig. 5).

The relationship between population fluctuations of pear lace bugs and average daily temperature changes was investigated using linear regression. The results showed that there is no significant relationship between the average daily temperature and population fluctuations in different life stages, except for the fifth nymph instar. Also, based on the linear regression method, it was found that there is no significant relationship between the fluctuation of *S. pyri* population and the average daily relative humidity in any of the biological stages.

Spatial distribution

The results of determining the spatial distribution of *S. pyri* population are presented in Table 3 and Table 4. Based on the results of both methods, the spatial distribution pattern was aggregated for all stages of life, and only a random pattern was recognized for the first instar nymphs using Iwao's method. An active aggregation was detected for all nymph instars based on the positive intercept values in Iwao's regression method. In contrast, the aggregation for adults was inactive due to a negative intercept value.

The relationship between the optimal sample size and the average density of various life stages of *S. pyri* was obtained at three precision levels of 10, 15, and 25% based on both Taylor's power law and Iwao methods (Fig. 6 & Fig. 7). To compare the two methods, Taylor's power law estimated a smaller sample size than Iwao at the same average population density. For example, at the mean total density of one insect per sample, the optimal sample size at the precision levels of 0.25, 0.15, and 0.1 was estimated as 28, 76, and 170 using Taylor's power law, and as 119, 329, and 740 using Iwao, respectively. Using lines to determine the number of optimal samples can be helpful in applied studies, especially in pest management. Pest management studies do not require high accuracy and a precision level of 0.25 is sufficient. However, in precise studies such as the preparation of field tables and the dynamics of populations, the optimal samples should be at levels of accuracy of 0.1 or at maximum of 0.15.

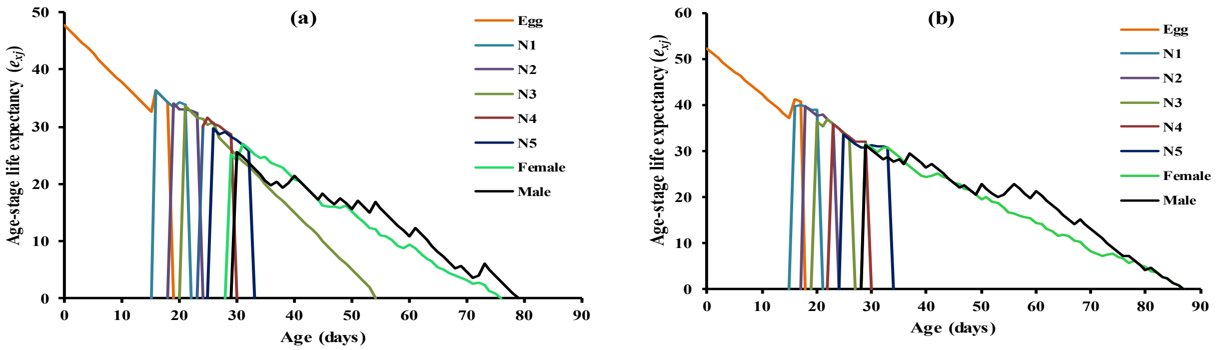


Fig. 4. Age-stage life expectancy (e_{xj}) of pear lace bug in laboratory (a) and field (b) conditions

Table 4. The results of spatial distribution of *Stephanitis pyri* population using Iwao's patchiness method

Life stage	Slope±SE	Intercept±SE	R^2	t_b	f	P_{value}
Nymph 1	4.420±5.313	2.391±0.640	0.103	0.643	0.692	0.437
Nymph 2	1.300±0.166	0.842±0.234	0.885	1.807	61.4	<0.001
Nymph 3	1.214±0.118	0.701±0.163	0.930	1.814	105.9	<0.001
Nymph 4	1.308±0.091	0.818±0.135	0.953	3.385	205.0	<0.001
Nymph 5	1.298±0.059	0.832±0.083	0.982	5.051	488.1	<0.001
Adult	1.144±0.121	0.241±0.071	0.909	1.190	90.0	<0.001

Discussion

To improve integrated pest management (IPM) tactics, access to different knowledge on insect-plant interactions including insect pest bioecology, population fluctuations and types of pest damage, and economic losses is incredibly desirable. Enhancing information about pest's status and different ecological factors related to them will lead to more successful and safer pest control (Flint & van den Bosch, 2012).

One of the essential sources of information for IPM is to know biological properties, in the specific demographics of a given population. The theory of age-stage, two sex life table includes both sexes and examines the growth and developmental variables for different biological stages of both sexes (Chi & Liu, 1985). A life table based on cohort gives us the most information about survival and reproduction in a population and is the basis of theoretical and practical ecology (Chi & Yang, 2003); therefore, we used the age-stage, two sex life table in this research. On the other hand, knowing population fluctuations in natural conditions can help us understand behavior and biology of pest and determine the factors influencing population changes.

So far, no information has been obtained about the biology and population fluctuations of *S. pyri* in the walnut trees. The present study can be described as the most comprehensive assessment of pear lace bugs in the field. More research has been done on a similar species called the Azalia lace bug, *Stephanitis pyrioides* (Scott), which lives in Europe and America.

Aysal and Kivan (2008), reported the biology of pear lace bugs at five temperatures on apple, in which the generation time at 26°C was 26.9±0.4 days. However, in the present study, the duration of one generation in a laboratory with a mean temperature of 25°C was 45.02 ± 0.52 days, which was significantly different from the previous investigation.

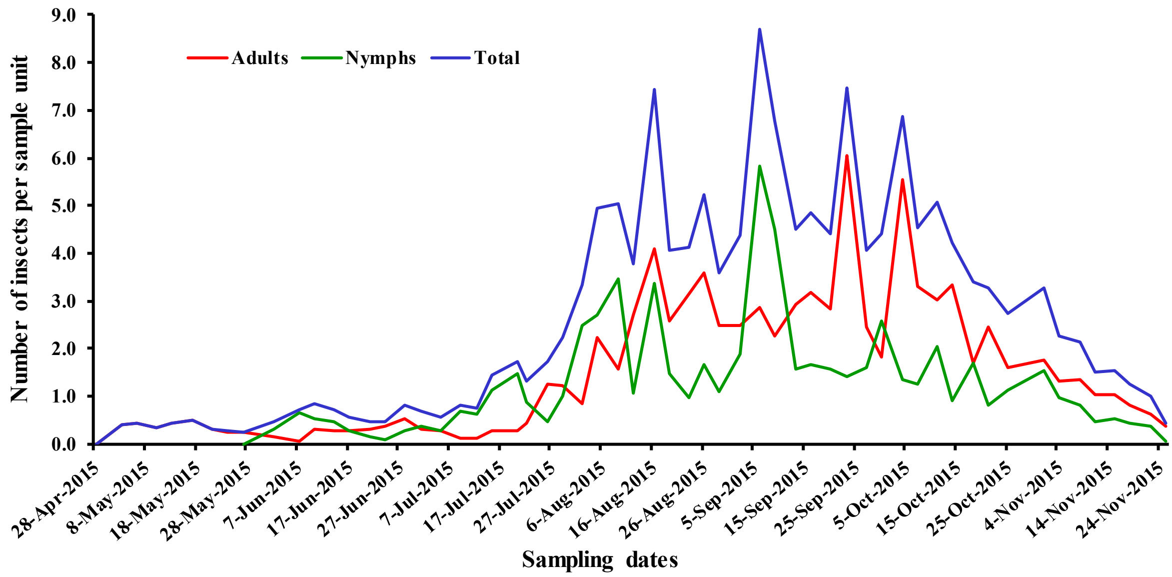
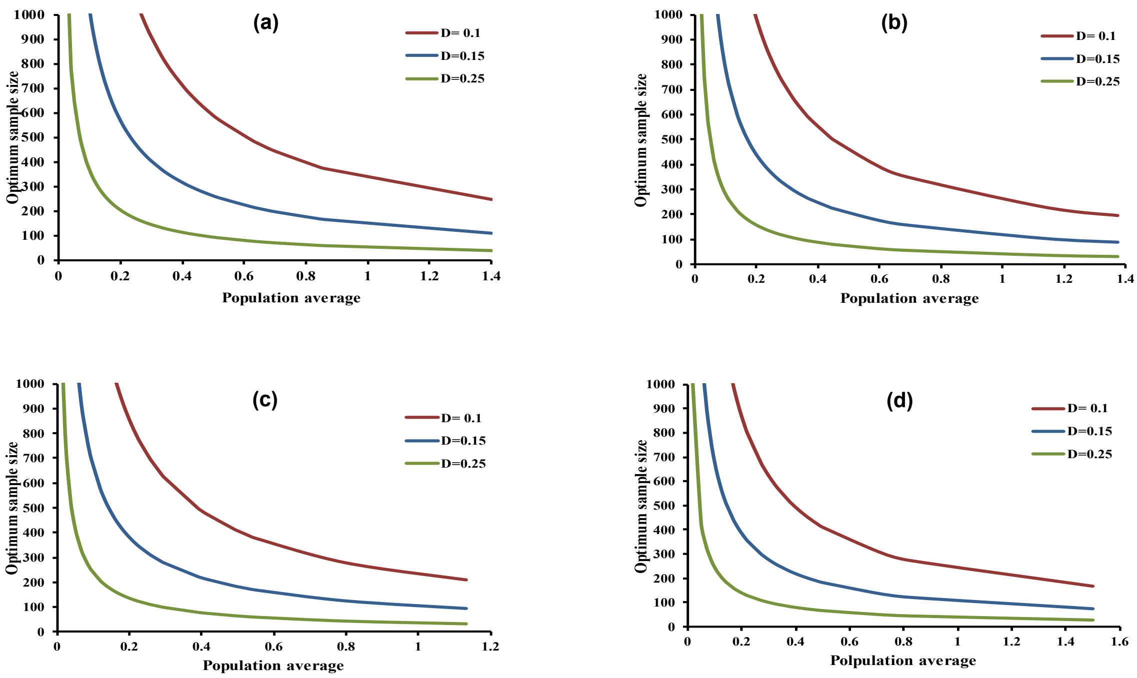


Fig. 5. Population fluctuation of adults, nymphs and total population of pear lace bug in 2015



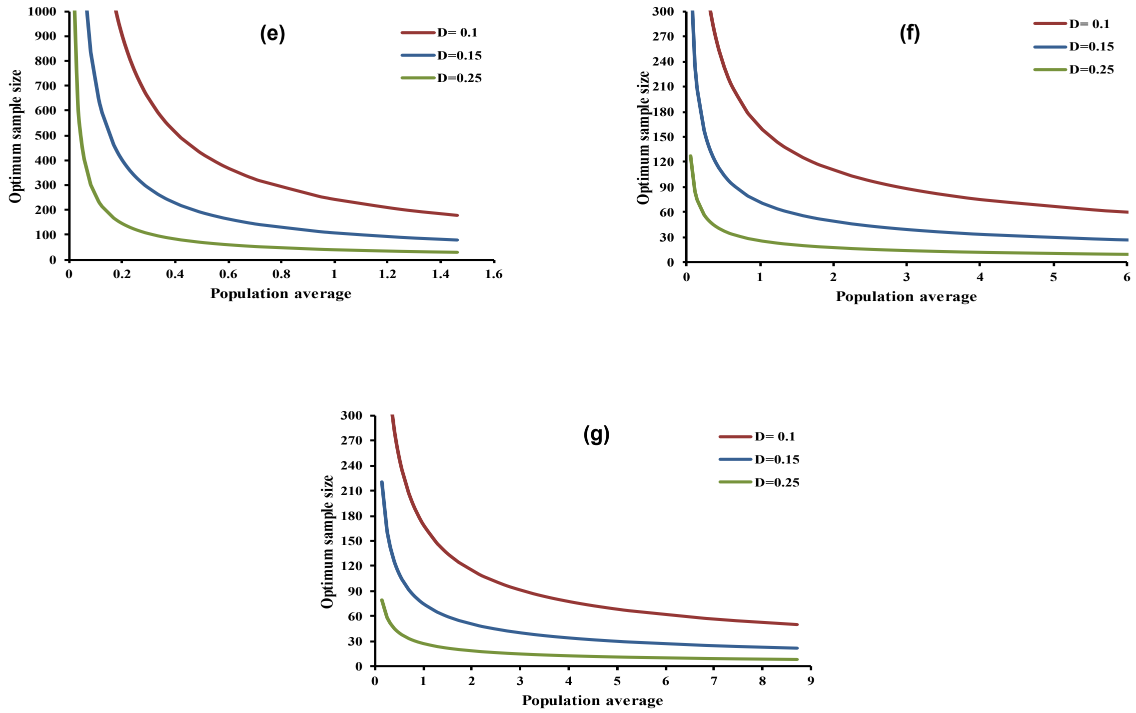
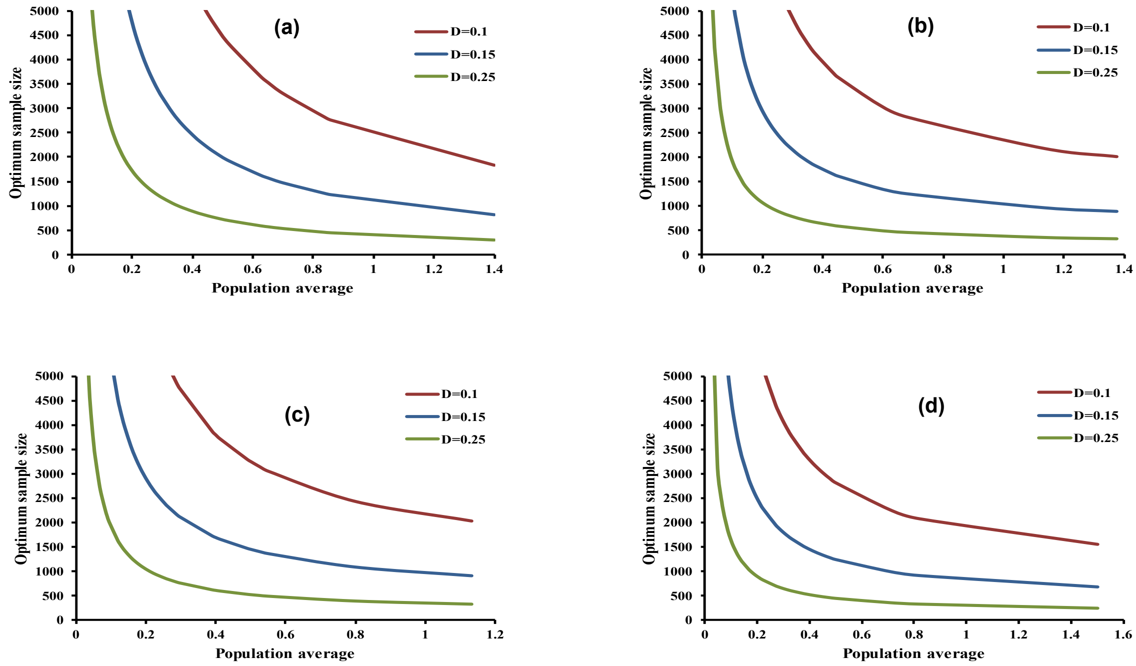


Fig. 6. The relationship between optimum sample size and the mean population density of N1(a), N2 (b), N3 (c), N4 (d), N5 (e), adult (f) and total population (g) of *Stephanitis pyri* using Taylor's power law

This difference can be due to the differences in plant hosts, different geographical conditions, and different adaptations of populations in these regions. Seasonal fluctuation of population of *S. pyri* and its related natural enemies have been surveyed in apple orchards of the West Azerbaijan (Akbarzadeh Shoukat, 2005). As a result, adult insects appeared on May 10, and their population density peaked in the last week of May.



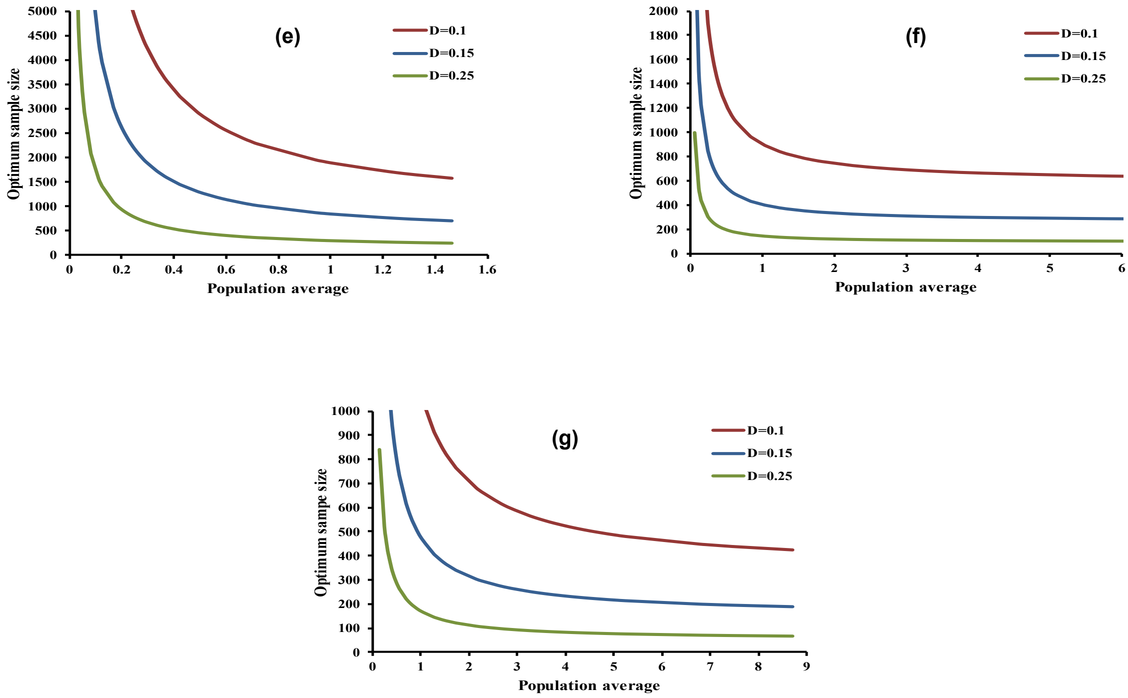


Fig. 7. The relationship between the number of optimal samples and the average population density of N1(a), N2 (b), N3 (c), N4 (d), N5 (e), adult (f) and total population (g) of *Stephanitis pyri* using Iwao's patchiness method

The egg-laying of overwintering adults began within a week after their emergence. The hatching of the eggs and the emergence of the first-generation nymphs starts from the middle of June, and the first peak of nymphs occurs in late July. The emergence of the first generation began in the last decade of June. At the beginning of summer, we found a mixed group of nymphs and adult insects in the population. Peaks of adults and population emerged about two weeks earlier in Kermanshah, compared to Western Azerbaijan province. It seems that this difference depends on the warmer climate of Kermanshah than West Azerbaijan. In both studies, pest overwintering was reported as adult insects, and population density in both regions was lower in the first generation but higher in later generations.

The study of the population density of *S. pyri* on 13 different apple varieties in the west of Turkey showed that there was no significant difference between population densities in different varieties and the growth of pest in apple trees stopped in the middle of October (Sahin *et al.,* 2009); however, in Kermanshah, the end of activity of the pear lace bug on walnut trees was delayed about one month and was observed in late November.

Despite the different conditions in the laboratory and the field, the life table parameters were so close that almost none of them had significant differences. The reason for this can be the excellent compatibility of the insect with different conditions, the suitability of the laboratory and field conditions for the growth and development of the insect, or other reasons that additional experiments can discover. By using the number of optimal sample graphs, given the specific density of pear lace bugs, it is possible to determine the number of samples required for optimal accuracy.

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چکیده

سنک گلابی، *Stephanitis pyri* (F.) (Hemiptera: Tingidae)، در سال‌های اخیر به عنوان یکی از آفات مهم درختان گردو در استان کرمانشاه مطرح شده است. اگرچه خسارت این آفت معمولاً اقتصادی نیست، ولی طغیان جمعیت آن می‌تواند موجب خسارت قابل توجه و حتی مرگ گیاه میزبان گردد. در این تحقیق، نوسانات جمعیت سنک گلابی روی درختان گردو، از اواخر زمستان تا اواخر پاییز فصل زراعی ۱۳۹۴–۱۳۹۵ در کرمانشاه بررسی شد. بر اساس داده‌های جمع‌آوری شده و با استفاده از روش‌های رگرسیونی تیلور و آیوائو، توزیع فضایی جمعیت آفت تعیین گردید. آماره‌های زیستی سنک گلابی در شرایط آزمایشگاهی و نیمه صحرایی مطابق با تئوری جدول زندگی سنی–مرحله‌ای دو جنسی تجزیه و تحلیل گردید. بر اساس نتایج حاصله، تفاوت معنی‌دار آماری بین هیچ یک از آماره‌های جدول زندگی (*r*: نرخ ذاتی افزایش جمعیت؛ R_0: نرخ خالص تولید مثل؛ λ: نرخ محدود افزایش جمعیت؛ *T*: میانگین زمان نسل و *GRR*: نرخ ناخالص تولید مثل) در شرایط آزمایشگاهی و نیمه‌صحرایی مشاهده نشد و بیش از ۷۰ درصد از جمعیت مورد مطالعه، دوره رشد و نموی پیش از بلوغ را با موفقیت سپری نمودند. سنک گلابی به صورت حشره کامل و روی علف‌های هرز دوره زمستان‌گذرانی را طی نمود و از اواخر اسفندماه، روی برگ‌های درختان گردو مهاجرت نمودند. یافته‌های تحقیق نشان داد که رابطه معنی‌دار آماری بین نوسانات جمعیت سنک گلابی و تغییرات میانگین دما و رطوبت نسبی روزانه وجود ندارد. توزیع فضایی جمعیت سنک گلابی بر اساس هر دو روش رگرسیونی تیلور و آیوائو، از نوع تجمعی تعیین گردید.

کلمات کلیدی: *Stephanitis pyri*، جدول زندگی، نوسانات جمعیت، توزیع فضایی

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