SPATIAL DISTRIBUTION OF THE COTTONY CAMELLIA SCALE, *PULVINARIA FLOCCIFERA* (WESTWOOD) (HEMIPTERA: COCCIDAE) IN THE TEA ORCHARDS

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Abstract: In the north of Iran, near the Caspian Sea, about 35627 hectares is cultivated with tea plant, *Camellia sinensis* on both plain and hilly land. The cottony camellia scale, *Pulvinaria floccifera* (Westwood) (Hemiptera: Coccidae) is one of the most important pests of tea orchards in the north of Iran. Spatial distribution is an important item in entomoecology and needs to be studied for many pest management programs. So, weekly sampling of *P. floccifera* population was carried out throughout the 2008-2010 season, in the tea gardens of the Tonekabon region of the Mazandaran province of Iran. Each cut branch of tea was determined as a sample unit and after primary sampling, sample size was calculated using the equation: $N= (ts/Dm)^2$, (D=0.15, sample size =50). The data acquired were used to describe the spatial distribution pattern of *P. floccifera* by Tylor's power law, Iwao's mean crowding regression, Index of Dispersion, and Index of Clumping. Tylor's power law ($R^2 > 0.84$) and Iwao's mean crowding regression ($R^2 > 0.82$) indicated that spatial distribution of 1st and 2nd nymphal instars is aggregated, but the distribution of 3rd instars, adults, and egg ovisacs is uniform. A result of *ID* and *IDM* showed that distribution of 1st, 2nd, and 3rd instars, adults, and egg ovisacs were aggregative each time the sampling was done.

Key words: Iran, *Pulvinaria floccifera*, spatial distribution, tea orchards

INTRODUCTION

A successful management of *P. floccifera* strongly depends on the development of an appropriate sampling plan (i.e. easy to implement, suitable for rapid decision making processes). In sampling programs, precision and cost-effectiveness are two of the most important factors that need to be considered (Pedigo 1994). For example, compared with fixed-sample size sampling, a fixed-precision sequential sampling can result in a 35-50% reduction in sampling effort (Binns 1994). The development of a sequential sampling scheme with a fixed statistical precision, therefore, may be useful for estimating *P. floccifera* density in tea orchards. Such an estimation, in turn, would be valuable for ecological and pest management studies. A sampling program can be used in ecological investigations (Faleiro *et al.* 2002), studies of population dynamics (Jarosik *et al.* 2003), when detecting pest levels that lead to a justification of control measures (Arnaldo and Torres 2005) as well as in assessing crop loss (Haughes 1996).

Many studies about spatial distribution of different species were made in other countries and on other crops, like the study of Nestel *et al*. (1995) with *Pulvinaria auranti* (Cock.) (Hom.: Coccidae), *Trialeurodes vaporariorum* (Westw) (Hom.: Aleyrodidae) and their natural enemies in Japan, *Parlatoria oleae* (Colvee) (Hom.: Diaspididae), and *Pseudococcus citri* Risso (Hom.: Pseudococcidae) in California (Tatara 1987), and *Pulvinaria regalis* Canard (Hom.: Coccidae) on chestnut in Germany (Sengonca and Feber 1996). Also, Geiger and Daane (2001) measured spatial distribution of *Pseudococcus maritimus* (Ehrhorn) (Hom.: Pseudococcidae) with Taylor's Index. Esfandiari and Mossadegh (2007) calculated spatial distribution of the cottony-cushion scale, *Icerya purchase* Maskell (Hom.: Margarodidae) on orange trees. Also Kozár *et al*. (2009) studied spatial distribution of homopteran pests and beneficial insects in an orchard and its connection with ecological plant protection. Loch and Zaluki (1996) evaluated spatial patterns of outbreaks of pink wax scale, *Ceroplastes rubens* Maskell (Hom.: Coccidae), within and among umbrella trees.

The most common methods employed to describe the patterns of dispersion of arthropod populations have been summarized by Southwood and Henderson (2000). Several estimates based on the dispersion coefficient, *k*, of the negative binomial distribution and on the relationship between variance and mean, are employed as indices of aggregation (Ludwig and Reynolds 1988; Krebs 1999; Southwood and Henderson 2000). Sampling plans based on these indices optimize the sampling effort as well as sampling precision (Kuno 1991). Sequential sampling plans are employed to more efficiently identify mean pest populations at or above the economic threshold. These plans have reduced the time required for sampling up to 50%, in comparison with conventional sampling plans (Pedigo and Zeiss 1996; Patrick *et al.* 2003). Although the objectives of sampling a finite population can differ, the development of a sampling procedure requires knowing the spatial distribution of populations (Liu *et al.* 2002).

In spite of the importance of *P. floccifera*, an efficient sampling program has not been developed nor has the spatial distribution been described. The objective of this study was to determine the spatial distribution patterns for *P. floccifera* nymphal stages and to develop and evaluate a fixed-precision sequential sampling for estimating the cottony camellia scale densities in tea orchards during two growing seasons.
The results can be employed to optimize the monitoring methods for establishing IPM strategies against the pest.

MATERIALS AND METHODS

Experimental Protocol

The studies were carried out in two tea orchards (a plain and a hilly region) in the suburbs of Tonekabon, Iran (36° 47'N, 50° 43'E, 44 m above sea level) from May to September, 2008-2010. In each orchard, sampling was done in an area with approximately 0.5 ha, containing tea plants (*Camellia sinensis* var. *sinensis*).

Development of sampling plans

An excised branch of tea tree was selected as the sample unit. From each tree, one branch was cut per week. In total, 25 samples were collected from each garden in each week. Sampling was done throughout the 2008-2010 season. The number of 1st and 2nd nymphal instars of *P. floccifera* was counted under a stereomicroscope. Relative variation (*RV*) was employed to assess the effectiveness of the sampling method. *RV* for the sampling data was calculated as follows: $RV=(SE/m)100$, where *SE* is standard error of mean and m is the mean of primary sampling data. A reliable sample size was determined using the following equation: $N=(ts/dm)^2$, where, N = sample size, t = t-student, s = standard deviation, d = desired fixed proportion of the mean, and m = the mean of primary data (Pedigo and Buntin 1994).

Spatial distribution pattern

The spatial distribution of *P. floccifera* among the collected sample units was determined by four commonly used methods: Iwao's patchiness regression, Taylor's power law, Index of Dispersion, and Index of Clumping.

Iwao's patchiness regression

This method was used to quantify the relationship between the mean crowding index (x^*) and mean density (m) using the following equation: $x^*=\alpha+\beta m$, where α indicates a tendency towards crowding (positive) or repulsion (negative), and β reflects the distribution of population in space and is interpreted in the same manner as b of Taylor's power law (Iwao and Kuno 1968). Theoretically, the mean crowding is the mean number of other individuals, per individual in the same quadrate: $x^*=m+(s^2/m-1)$

As with the variance-to-mean ratio, the index of patchiness is dependent upon quadrate size β=1 random, <1 regular and >1 aggregated (Lloyd 1967).

Taylor's Power Law

For many insect and animal species, Taylor (1961) found that a power law function could be used to model the relationship between mean and variance as: $s^2=am^b$, where s^2 is the variance; m the sample mean; a is a scaling factor related to sample size and b measures the species aggregation. When b= 1, <1 and >1, the distribution is random, regular and aggregated, respectively. Through use of a log transformation, one can estimate the coefficients with linear regression as: $\log(s^2)=\log(a)+b\log(m)$, where a and b are the parameters of the model, estimated by linearizing the equation after a log-log transformation (Taylor 1961).

The student's *t*-test can be used to determine whether the colony is composed of single individuals, and to determine if colonies are dispersed randomly. Test b=1: $t=(b-1)/SE_b$ and Test β=1: $t=(\beta-1)/SE_\beta$, where SE_b and SE_β are the standard errors of the slope for the mean crowding regression.

Calculated values are compared with tabulated *t*-values having n-2 degrees of freedom. If the calculated t (t_c) < t-table (t_t), the null hypothesis (b=1) would be accepted and spatial distribution would be random.

If $t_c > t_t$, the null hypothesis would be rejected and if b > 1 and b < 1, the spatial the spatial distribution would be aggregated and uniform, respectively.

Index of dispersion

Dispersion of a population can be classified through a calculation of the variance to mean ratio; namely: s^2/m=1 random, < 1 regular and > 1 aggregated. Departure from a random distribution can be tested by calculating the index of dispersion (I_D), where n denotes the number of samples:

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

I_D is approximately distributed as x^2 with n-1 degrees of freedom. Values of I_D which fall outside a confidence interval bounded 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 (Southwood 1995).

Index of Clumping

The Index of Clumping suggested by David and Moore (1954):

$$IDM = \frac{S^2}{\bar{x}} - 1$$

When $IDM = 0$, < 0 and > 0, the distribution is random, regular, and aggregated.

Optimum number of sample units (sample size)

The optimum sample size is the smallest number of sample units that would satisfy the objectives of the sampling program and achieve the desired precision of estimates. Finding out the Taylor power law and Iwao's regression coefficients eliminates the experimental needs for a large sample size (Ifoulis and Savopoulou-Soultani 2006). The optimum number of sample units was derived from a formula using Taylor's power law coefficients:

$$n_{opt} = \left(\frac{t_{\alpha/2}}{D}\right)^2 a\bar{x}^{-b-2}$$

and using Iwao's regression method coefficients (Buntin 1994; Young and Young 1998):

$$n_{opt} = \left(\frac{t_{\alpha/2}}{D}\right)^2 \left(\frac{\alpha+1}{\bar{x}} + (\beta - 1)\right)$$

where D represents the range of accuracy, a and b is Taylor regression coefficients, α and β is Iwao's equation coefficients, m is mean density of populations, and t α/2 is t-student. The optimum number of sample units with 15, 20, and 30% confidence interval levels have been calculated and plotted using Microsoft Excel.

RESULTS

Sampling program

The results from primary sampling showed reliable sample size with a maximum variation of 10% and 20% using Taylor's power law and Iwao's patchiness regression indices. Using these data, the relative variations (RV) obtained for each of the hilly and plain gardens, were 7.24%, 11.4%, 8.3%, and 9.85%, respectively, all were less than 25% and were acceptable. To increase accuracy for determining the sample size, a 20% error rate was placed in the formula and the number of samples was determined as 14, 29, 19, and 26 samples (tea shoots), respectively. According to the sampling method and coordination with sampling in gardens at each sampling date, 50 samples were taken from each orchard.

Spatial distribution of *P. floccifera*

Taylor's Power Law

The index amounts with confidence interval for plain and hilly gardens, describe the spatial distribution of *P. floccifera* in the 2009 season (Table 1). The index amounts show that R^2 >0.82 in all cases, and the amounts describe the spatial distribution of 1st and 2nd nymphal instars of *P. floccifera* nicely (Table 1). In all cases $b>1$, therefore, they have aggregated spatial distribution.

The confidence interval (95%) of b index was not included in any case.

The regression line's gradient of 2nd nymphal instar is *b>1*; only in the hilly garden was it less than one. The results show that although the distribution was random on only one occasion, there is a pattern of aggregated distribution for these instars.

The index amounts (95%*CI*) of hilly and plain gardens describe the spatial distribution of *P. floccifera* in the 2010 season (Table 2). With respect to the amount of b (95%*CI*) and calculating t_b, the spatial distribution of 3rd nymphs, adults, and ovisacs in all hilly and plain gardens was uniform (R^2 > 0.83). The ovisacs did not show significant differences. The spatial distribution of 1st instar nymphs in all hilly and plain gardens was aggregated but for 2nd instar nymphs in the first hilly garden, the spatial distribution was only aggregated, and in the other garden, it was uniform (Table 2).

The distribution descriptive charts of *P. floccifera* in different growth stages in hilly and plain gardens, with Taylor's power law are shown in Figure 1. The dotted line is random spatial distribution and drawn for comparison. If the regression line is over, down or on the dotted line, then the spatial distribution is aggregate, uniform, and random, respectively.

Iwao's patchiness regression indices

Amounts of α and β (95% *CI*) for hilly and plain gardens describe the spatial distribution of *P. floccifera* in the 2009 season (Table 1). According to this Table, spatial distribution of 1st and 2nd instar nymphs is aggregative in plain gardens and in the second hilly garden. In all cases, the amount of *β* is higher than one. The amount of β in the first hilly garden is more than one, but *CI* 95% (confidence interval) contains the one. The calculated t_b shows that β and one do not have significant differences and the spatial distribution of 1st and 2nd instar nymphs is uniform. The sum total of 1st and 2nd instar nymphs showed aggregated spatial distribution in all gardens (R^2> 0.93).

The amounts of α and β (95% *CI*) for hilly and plain gardens described the spatial distribution of *P. floccifera* in the 2010 season (Table 2). According to this Table, the amount of *β* is higher than one but there is not a significant difference between *Lloyd's* mean crowding and the population means of 3rd instar nymphs, adult insects, and ovisacs in plain gardens, as well as adult insects and ovisacs in the second hilly garden.

The 1st instar nymphs have an aggregate spatial distribution in all the hilly and plain gardens, but 2nd instar nymphs show this condition in the second plain garden only. The spatial distribution is uniform in other gardens. The data of Table 2 confirms this information.

The descriptive distribution charts of *P. floccifera* in different growth stages, with Iwao's patchiness regression in hilly gardens, are shown in Figure 2.

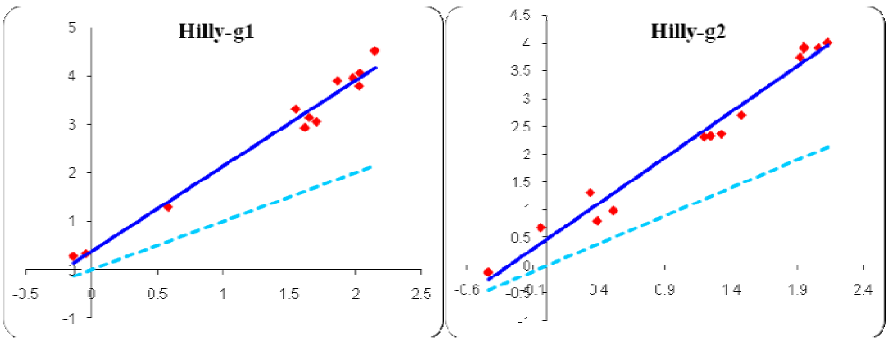
The dotted line is random spatial distribution and drawn for comparison. The spatial distribution of different instars and different sampling occasions followed the aggregate pattern, except for the second instar (Hilly-g2) in which the distribution was random. Later, in the discussion, we seek an explanation for this difference.

Table 1. Spatial distribution of *P. floccifera* in plain and hilly tea gardens during the 2009 season, using Taylor's power law and Iwao's patching regression analysis in the Tonekabon region of the Mazandaran province of Iran.

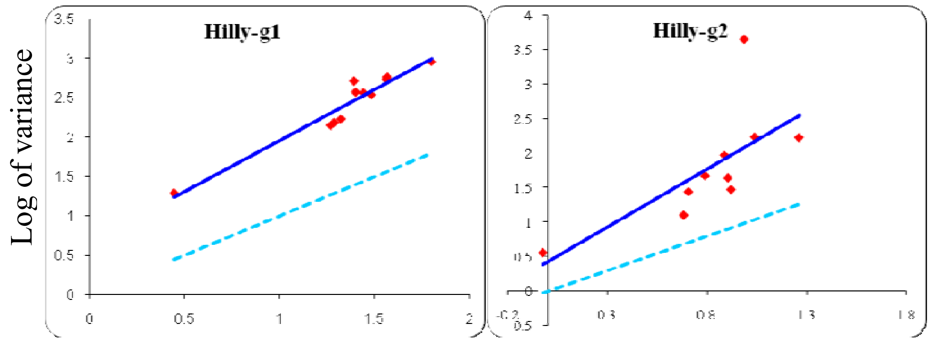
Area	Garden	Development Stage	Taylor						Iwao					
			a	b	SE_b	r^2	P_{reg}	t_c	α	B	SE_β	r^2	P_{reg}	t_c
Plain	First	1st instar	0.47	1.54	0.10	0.97	0.000	5.40	2.85	1.37	0.09	0.97	0.000	4.11
		2nd instar	0.39	1.33	0.13	0.94	0.000	2.54	1.15	1.25	0.07	0.98	0.000	3.57
		Sum	-0.47	2.04	0.10	0.97	0.000	10.4 0	-1.42	1.43	0.07	0.97	0.000	6.14
	Second	1st instar	0.48	1.46	0.06	0.98	0.000	7.66	3.39	1.26	0.06	0.98	0.000	4.33
		2nd instar	-1.52	3.07	0.30	0.95	0.000	6.90	-11.00	2.35	0.28	0.93	0.000	4.82
		Sum	-0.03	1.79	0.19	0.89	0.000	4.16	8.38	1.2	0.08	0.95	0.000	2.50
Hilly	First	1st instar	0.66	1.36	0.06	0.99	0.005	6.00	9.85	1.11	0.05	0.98	0.000	2.20
		2nd instar	0.65	0.98	0.20	0.83	0.000	-0.10	3.08	1.02	0.08	0.97	0.000	0.25
		Sum	-0.19	1.77	0.15	0.93	0.000	5.13	6.08	1.14	0.05	0.98	0.000	2.80
	Second	1st instar	0.55	1.54	0.11	0.96	0.000	4.90	5.72	1.38	0.09	0.97	0.000	4.22
		2nd instar	-0.53	2.22	0.46	0.85	0.009	2.62	-9.67	2.05	0.24	0.93	0.000	4.37
		Sum	-0.18	1.95	0.18	0.92	0.000	5.27	6.18	1.38	0.09	0.95	0.000	4.22

Table 2. Spatial distribution of *P. floccifera* in plain and hilly tea gardens during the 2010 season, using Taylor's power law and Iwao's patching regression analysis in the Tonekabon region of the Mazandaran province of Iran.

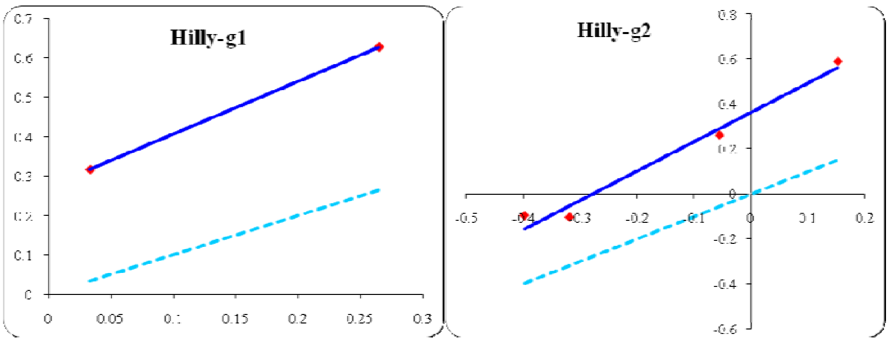
Area	Garden	Development Stage	Taylor						Iwao					
			a	b	SE_b	r^2	P_{reg}	t_c	α	B	SE_β	r^2	P_{reg}	t_c
Plain	First	3rd instar,	0.14	0.83	0.10	0.97	0.015	-1.7	0.97	0.12	0.39	0.05	0.788	-2.25
		Adult	0.45	1.27	0.24	0.93	0.035	1.12	0.28	0.36	3.12	0.41	0.361	-0.20
		Ovisac	0.59	1.9	0.54	0.86	0.071	1.66	-1.77	6.40	3.19	0.66	0.184	1.69
		1st instar	0.50	1.58	0.11	0.95	0.000	5.27	0.78	1.80	0.10	0.96	0.000	8.00
		2nd instar	0.74	1.07	0.14	0.88	0.000	0.50	4.85	1.07	0.11	0.92	0.000	0.63
	Second	3rd instar,	0.26	1.10	0.06	0.99	0.003	1.66	0.43	1.55	0.41	0.87	0.063	1.34
		Adult	0.12	1.00	0.17	0.94	0.029	0.00	0.07	3.62	2.80	0.63	0.410	0.93
		Ovisac	0.43	0.18	0.37	0.84	0.085	-2.2	1.20	1.52	1.92	0.24	0.511	0.27
		1st instar	0.59	1.38	0.07	0.97	0.000	5.42	1.82	1.44	0.08	0.96	0.000	5.50
		2nd instar	0.70	1.20	0.14	0.89	0.000	1.42	3.92	1.32	0.11	0.94	0.000	2.90
Hilly	First	3rd instar,	0.14	0.67	0.01	0.99	0.007	-33	0.64	0.71	0.01	0.99	0.005	-29.0
		Adult	0.02	0.45	0.03	0.99	0.042	-18	1.73	-0.88	0.28	0.91	0.092	-6.71
		Ovisac	0.23	1.04	0.23	0.92	0.044	0.17	0.56	1.17	0.35	0.85	0.071	0.48
		1st instar	0.40	1.76	0.08	0.98	0.000	9.5	-10.32	2.22	0.22	0.91	0.000	5.54
		2nd instar	0.25	1.53	0.07	0.96	0.000	7.57	7.22	1.15	0.08	0.95	0.000	1.87
	Second	3rd instar,	0.36	1.30	0.14	0.98	0.012	2.14	0.40	1.88	0.30	0.95	0.024	2.93
		Adult	1.74	1.74	0.23	0.96	0.017	3.21	-0.73	2.64	0.17	0.99	0.004	9.64
		Ovisac	2.26	2.23	0.36	0.95	0.025	3.41	-10.53	8.52	4.13	0.52	0.008	1.82
		1st instar	0.47	1.64	0.08	0.97	0.000	8.00	2.49	1.74	0.08	0.97	0.000	9.25
		2nd instar	0.44	1.67	0.62	0.48	0.027	1.08	15.76	5.50	1.96	0.04	0.062	2.30



a- The log of mean population density of 1st instar nymphs

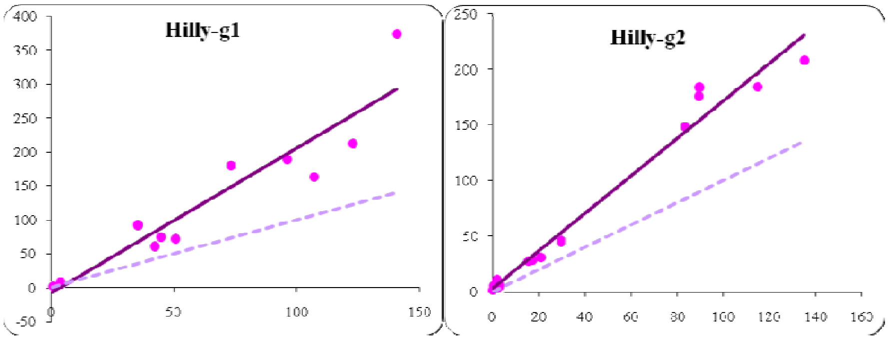


b- The log of mean population density of 2nd instar nymphs

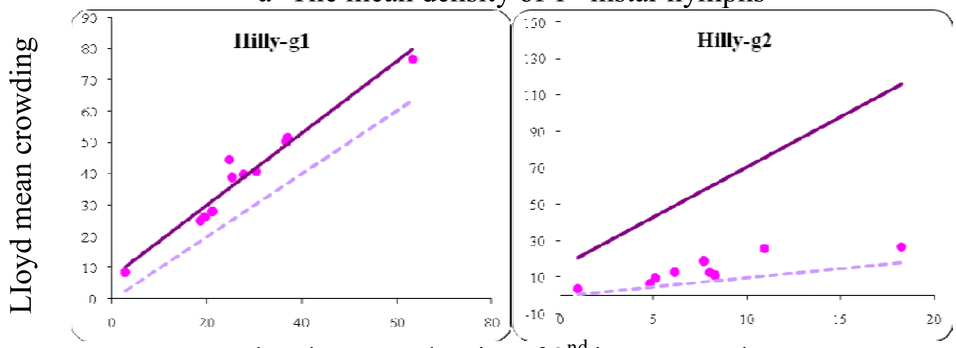


c- The log of mean population density of 3rd instar nymphs

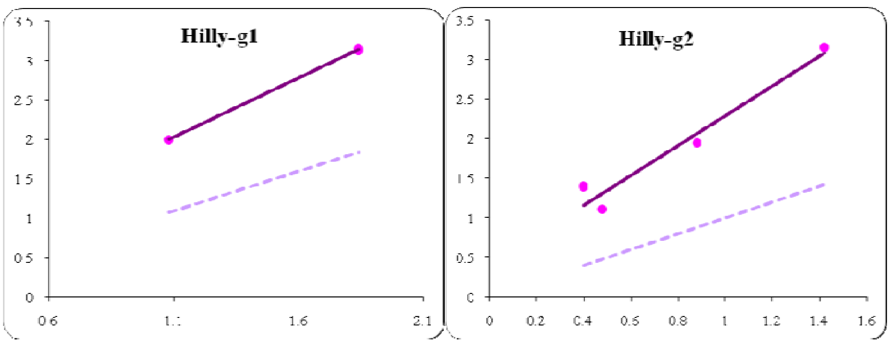
Fig. 1. A regression line between log(s^2) and log(m) of 1st, 2nd, and 3rd instar nymphs of *P. floccifera* in hilly gardens in 2010. The dotted line is random spatial distribution and drawn for comparison. If the regression line is over, down or on dotted line, then spatial distribution is aggregate, uniform, and random, respectively.



a- The mean density of 1st instar nymphs



b- The mean density of 2nd instar nymphs



c- The mean density of 3rd instar nymphs

Fig. 2. A regression line between the *Lloyd* mean crowding (x^*) and mean density (m) of 1st, 2nd. and 3rd instar nymphs of *P. floccifera* in hilly gardens in 2010. The dotted line is random spatial distribution and drawn for comparison.

Spatial distribution with ID and IDM

The calculated amount of *ID* and *IDM* are shown in Table 3 for the population density of *P. floccifera* in different growth stags in the 2009-2010 seasons. The amounts of *ID* and *IDM* for 1st and 2nd instar nymphs showed that the spatial distributions in hilly and plain gardens were aggregate in the two years.

The spatial distribution of 3rd instar nymphs, adult insects, and ovisacs was aggregate every day, and uniform sometimes, or random infrequently. Taylor's Power Law and Iwao's patchiness regression indices showed that 3rd nymphal instars have aggregated spatial distribution in all their developmental stages.

***Optimum number of sample units* (*Sample size*)**

Figure 3 shows the relationship between the sample size and mean of 1st, 2nd, and 3rd instar nymphs, adult insects and ovisacs in hilly gardens in the 2009 season. This figure shows two attention levels (10% and 20%) by Taylor's Power Law, Iwao's patchiness regression indices, and general formula.

Table 3. Spatial distribution of *P. floccifera* in plain and hilly tea gardens during the 2009-2010 season, using *ID* and *IDM* indices in the Tonekabon region of the Mazandaran province of Iran.

Index	Area	Garden	Development stage				
			1st star	2nd star	3rd star	Adult	Ovisac
ID	Hilly	First	2120.2	452.2	58.4	77.8	84.7
		Second	1754.2	564.5	105.2	112.4	735.1
	Plain	First	1066.1	329.0	80.6	80.1	161.7
		Second	1528.7	396.7	79.0	72.9	135.9
IDM	Hilly	First	42.2	8.2	0.89	0.58	0.7
		Second	34.8	34.9	1.2	1.4	14
	Plain	First	19.33	5.7	0.95	0.7	2.3
		Second	12.9	7.1	0.91	0.9	1.5

X^2 for α=0.05, 0.95 is 67.5, 34.76; df =49

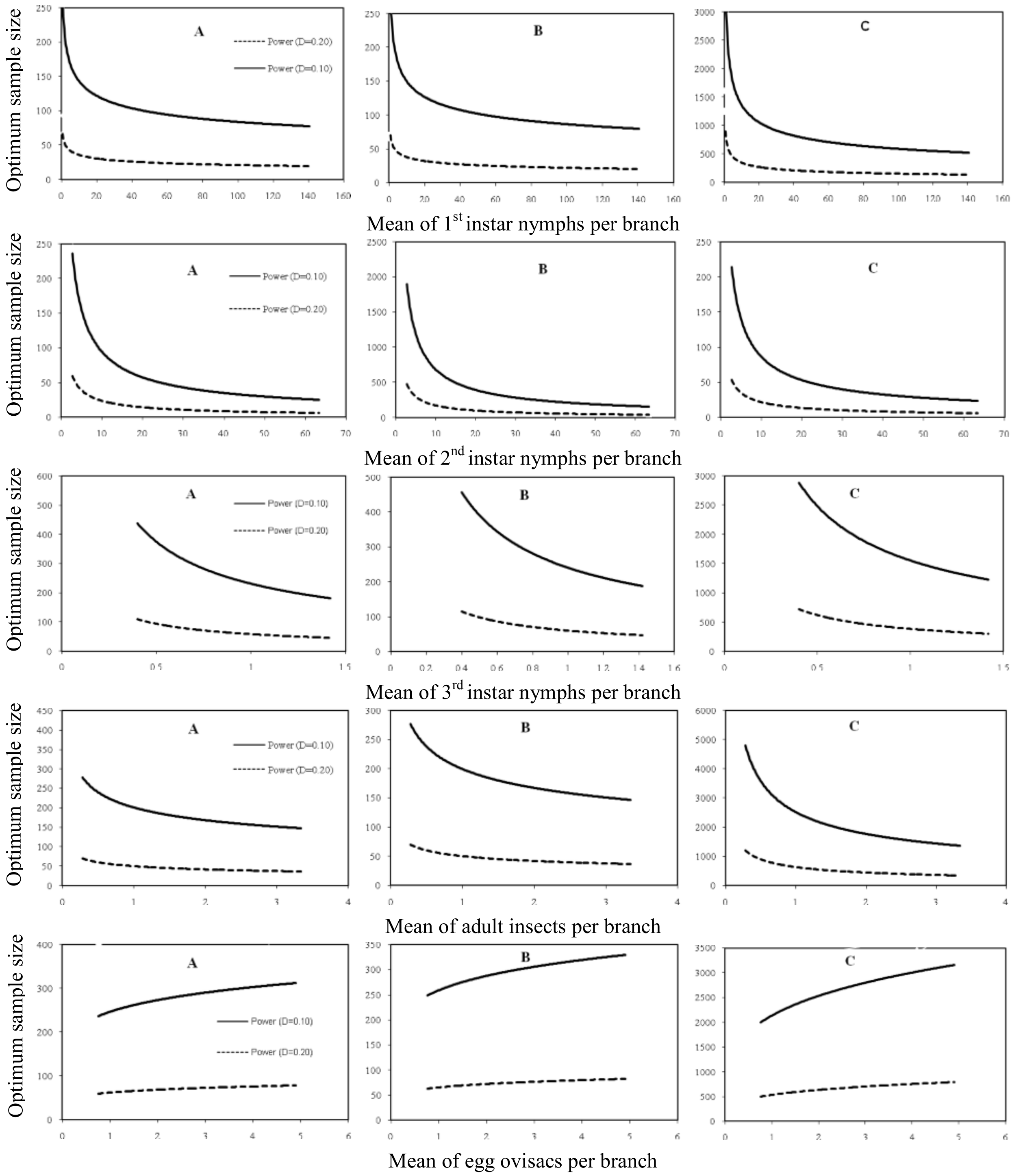


Fig. 3. The relationship between the mean of different stages of *P. floccifera* per branch, in two attention levels of general formula (A) Taylor's Power Law regression index (B) Iwao's patchiness regression index (c).

DISCUSSION AND CONCLUSIONS

Hallaji sani (2006) studied the spatial distribution of *P. auranti* in citrus gardens in Mazandaran using Taylor's Power Law and he found that it was aggregated as we found it for *P. floccifera*. The spatial distribution of *P. oleae* and pine scale was discerned aggregate (Nestel *et al*. 1995), which is also similar to our results. The amount of *b* in plain gardens in the 2010 season was less than in 2009, while in hilly gardens it was the reverse. The amount of *b* may change in different environmental conditions, places, and times, but the result is constant. The amount of 1st instar nymphs in plain gardens was less than in hilly gardens in 2008, and the calculated amount in hilly gardens was more than in plain gardens in 2009. But the spatial distribution of 1st instar nymphs is aggregated.

The adult insects had an aggregated spatial distribution, usually as a result, the ovisacs also have an aggregated spatial distribution. The spatial distribution of 2nd instar nymphs was aggregate or uniform. Therefore, insects can begin life with a specific spatial distribution, have a different spatial distribution during their life, and finish with another type. The insects cannot have one type of specific spatial distribution for all their life stages (Rajabi 2003).

The spatial distribution of 1st, 3rd, adult insects, and ovisacs displayed similar result as Taylor's Power Law and Iwao's patchiness regression indices in hilly and plain gardens in the 2009 and 2010 seasons. The spatial distribution of 2nd instar nymphs showed that insect spatial distribution can be dissimilar in different places, as the spatial distribution of 2nd instar nymphs was dissimilar in hilly and plain gardens, and as was true even in the first and second hilly gardens. For example, the 2nd instar nymphs have a uniform spatial distribution in the first hilly garden but have an aggregate spatial distribution in the second hilly garden in 2009.

The result of this study is in accordance with the explanation of Rajabi (2003) regarding spatial distribution of insects. The results of Meagher *et al.* (1996) showed that spatial distribution of *Eoreuma loftini* (Dyar) (Lep.: Pyralidae) small larva is aggregate, while distribution of medium and large larvae is uniform. The result of this research showed that with the increase of nymphal instars and their bodies, aggregated spatial distribution becomes uniform. This is natural and instinctive for survival protection. Because 1st instar nymphs are first and at a sensitive stage, they stay next to each other, and make a defensive bumper.

These results are functional for insect ecology and behavior research that have similar samples. The comparison between different methods shows that minimum sample size is calculated by general formula, and maximum sample size is calculated by Iwao's patchiness regression indices. Golizadeh (2006) indicates that sample size calculated by Taylor's Power Low indices was lower than sample size calculated by Iwao's patchiness regression indices.

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