Dispersion pattern and fixed precision sequential sampling of *Sitobion avenae* (Fabricus) (Hemiptera: Aphididae) in wheat fields of Badjgah (Fars province) in Iran

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Abstract

Understanding the dispersion pattern of a species is an important pre-requisite for developing an effective pest management program. In this study, four hundred wheat plants were surveyed for *Sitobion avenae* twice a week during 2010 and 2011 growing seasons in two fields of Badjgah (Fars province) in Iran. In each field only one of the two cultivers of Bahar or Shiraz was planted. Analysis of spatial distribution pattern using Taylor's power law and Iwao's regression model showed that *S. avenae* exhibited an aggregated distribution on wheat. Taylor's power law was estimated from 84 data sets and fitted the data better than Iwao's regression model. The optimal sample sizes needed for fixed precision levels of 0.25 and 0.30 were estimated using Taylor's regression coefficients, and the required sample sizes increased dramatically with increased levels of precision. Therefore, the sampling-plan we presented here should be used as a tool for an efficient estimation of *S. avenae* population density in wheat fields for pest management decision.

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Key words: spatial distribution, *Sitobion avenae*, Taylor's power law, sequential sampling, Iwao's regression model.

Acknowledgments: we would like to express our sincere gratitude to Dr. Mohiseni for generous assistance with various aspects of this project. This study was supported by the grants from Shiraz University, Iran.

Received for publication: 14 July 2013.
Revision received: 13 October 2013.
Accepted for publication: 16 October 2013.

Journal of Entomological and Acarological Research 2013; 45:e22
doi:10.4081/jear.2013.e22

Introduction

Approximately 463,000 ha of winter wheat - *Triticum aestivum* L., are annually planted in Fars province (Iran) annually (Kherad, 2013). The English grain aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) is regarded as one of the most important aphids of cereals in this region (Hodjat & Azmayesh Fard, 1986) and causes damage by sap feeding it is also a vector of barley yellow dwarf virus (BYDV) which may result in significant yield losses (Williams & Wratten, 1987).

Feeding by adult and nymphs of *S. avenae* before the flowering stage can result in reduceing the number of grains in the ear. After flowering to the end of grain filling, it reducing directly the size of the grain (Hodjat & Azmayeshfarrd, 1986). This species is more cold - hardy than *R. padi*, and thus has a more significant role in the secondary spread of BYDV in winter cereals (Williams & Wratten, 1987).

Dispersion and abundance of organisms are the most important properties of insect population and essential ecological properties of species (Siswanto *et al*., 2008). Knowledge about dispersion pattern of an organism is required for understanding population biology, resource exploitation and dynamics of biological control agents (Fauvergue & Hopper, 1994). It provides a better understanding of the relationship that exists between organism and its environment which may be helpful in planning efficient sampling programs for population estimates, development of population models and pest management strategy (Soemargono *et al*., 2008).

There are many methods used to describe the dispersion of arthropod populations, but most estimates are based on sample means and variances (Bisseleua *et al*., 2011), while the relationships between the variance and mean are used as indices of aggregation (Arnaldo & Torres, 2005). The models of Taylor and Iwao also depend on the relationship between the sample mean and the variance of insect numbers per sampling unit. The slope of the regression model is used as an index of aggregation. Designing sampling plans based on these indicators has been reported to reduce sampling effort, cost and minimize variation of sampling precision (Kuno, 1991; Payandeh *et al*., 2010).

Despite the fact that Fars province has the first rank of wheat production in Iran (Kherad, 2013), and economic importance of *S. avenae* to wheat growers, little is known about its dispersion in Iran. Thus, there is an urgent requirement for such information as it will provide wheat pest managers, researchers, and farmers with a cost-effective sampling method for *S. avenae*. Therefore, this study was undertaken to determine dispersion pattern of *S. avenae* in order to develop a suitable sampling plan for the pest.

Materials and methods

Study site and population sampling

The study was carried out from March 2010 to June 2011 at two pesticide- free rectangular wheat fields at Badjgah region, Fars province (N 52'42" E 29'50"). Each field has an area of 2 hectares. In each field, one of the two cultivars, Shiraz and Bahar, were planted separately and agronomic practices, such as application of manure, were given to wheat fields at regular intervals. The fields were sampled 2 days per week throughout the growing season (from initiation of tillering till grain ripening stage), unless rainfall increased intervals between sampling dates. Tillers were collected by traveling a X-shaped procedure and the data from primary sampling were then used to develop sample size for the English grain aphid using formula (1) described by Karandinos (1976):

$$N = \left(\frac{Z_{\alpha/2}}{D}\right)^2 . \left(\frac{S}{m}\right)^2 \tag{1}$$

where N is the number of samples (each sample contains 5 tillers), D is the precision level, $z_{\alpha/2}$ is the value of z distribution for the desired significance level (in our case α= 0.1), S^2 and m are variance and mean respectively.

Determination of the appropriate sample unit

Then, the most appropriate sample unit was estimated by calculating the relative variation (RV) using formula (2):

$$RV = \frac{SE}{mean} * 100 \tag{2}$$

Sampling efficiency also was calculated as the relative net precision (RNP) using formula (3):

$$RNP = \frac{100}{(RV * Cs)} \tag{3}$$

where RV, SE, and Cs are the relative variation, Standard error of mean, and the cost in minutes to count aphid abundance on an individual sample unit, or mean search time (Pedigo *et al*., 1972; Karandinos 1976; Zar, 2010 missing in ref list; Hall *et al*., 1991; Buntin, 1994).

Taylor's power law

Taylor's power law (TPL) discribes the regression between logarithm of population variance and logarithm of population mean according to the following equation:

$$Log(S^2) = Log(\alpha) + bLog(\bar{x}) \tag{4}$$

where S^2 is the population variance, $\bar{x}$ is the population mean, α is the Y-intercept and *b* is the slope of the regression line;. *b*<1, *b*=1 and *b*>1 indicate uniform, random and aggregated spatial patterns, respectively (Southwood, 1978; Taylor, 1984; Davis, 1994).

Iwao's method

The Iwao's patchiness regression method quantifies the relationship between the mean crowding index (m*) and the mean (m) by the following formula:

$$m^* = \alpha + \beta m \tag{5}$$

where m was determined as $[m(S^2/m\text{-}1)]$. The intercept (α) is the index of the basic component of a population or basic contagion (where α<1, α=1, and α>1 represent regularity, randomness, and aggregation of populations in spatial patterns, respectively), and the slope (β) is the density contagiousness coefficient interpreted in the same manner as *b* of Taylor's regression (Sule *et al*., 2012).

Test for significant difference between regression coefficients (*b* index) from 1 was calculated by the following formula:

$$t = (slope - 1)/SE_{slope} \tag{6}$$

where *slope* and *SE slope* were Taylor's coefficient and its standard error in Regression equations, respectively. The amount of calculated *t* was compared with *t* value given in the table, the degrees of freedom is (N–1). If the absolute value of calculated *t* was greater than the value given in the table, then the spatial distribution of the aphid was aggregation (Feng & Nowierski, 1992).

Presence or absence of difference between cultivars were calculated based on formula (7) with (N_1+N_2)-2 degrees of freedom (Feng & Nowierski, 1992):

$$t_{slope} = (b_1 - b_2) / \sqrt{(SE_{b_1}^2 + SE_{b_2}^2)} \tag{7}$$

where b_1 and b_2 were Taylor's coefficient of two cultivars and SE_1 and SE_2 were their standard errors.

Constructing fixed percision sampling schemes

Based on the sample counts, the optimal sample sizes (n) was calculated with *a* and *b* from Taylor's Power Law to develop the enumerative sampling plan by Green (1970), with precision levels of 0.15, 0.25 and 0.3 for ecological and pest management purpose, as recommended by Green (1970), using the following formula:

$$n = \frac{\alpha \bar{x}^{b-2}}{D_{\exp}^2} \tag{8}$$

where n is the number of sample unit required to estimate the mean number of aphids, *D* is a desired precision and *a* and *b* are the Taylor's Power Law coefficients. The sampling stop line was calculated as suggested by Elliott *et al.* (2003) using the following formula:

$$T_n = \left(\frac{D_{\exp}^2}{\alpha}\right)^{1/(b-2)} n^{(b-1)/(b-2)} \tag{9}$$

where Tn is the cumulative number of aphids in a sample of n sample units and defines the sequential sampling stop line. Sample size curves and sequential sampling stop lines were generated by a computer program in Excel. The coefficients of Taylor's Power Law were estimated by linear least square regressions using PROC REG (SAS, 1999) on the linearized version of TPL. Using Green's method, the resampling for validation of sampling plans (RVSP) program was used to validate the sequential sampling plans of *S. avenae* (Naranjo & Hutchison, 1997; O'Rourke & Hutchison, 2003). RVSP requires the use of independent data sets for validation. Thus, 15 data sets representing a range of low, medium, and high densities were selected at random from both the 84 *S. avenae* data sets to serve as validation data sets. Resampling was repeated 500 times for each data set, producing the average, minimum and maximum precision level and the average, minimum and maximum sample size (Naranjo & Hutchison, 1997). Then, the numbers of samples in conventional method and Green's method were compared (Shahrokhi & Amir-Maafi, 2011; Mohiseni *et al*., 2009).

Wilson and Room's model

To describe the relationship between the proportion (p) of sampling units (tillers) with >0 *S. avenae* individuals and the mean number of individuals per sampling unit, the equation of Wilson & Room (1983) was used:

$$P(I) = 1 - e^{-\overline{x} ln(a.\overline{x}^{b-1})(a.\overline{x}^{b-1}-1)^{-1}} \quad (10)$$

where a and b are Taylor's estimates. This P(I) equation can be used for predicting the mean number of individuals of a given species per sampling unit ($\overline{x}$) from a simple count of the proportion of sampling units in which this species is present (p).

Results

Determination sample size and sample unit

In all cases, levels of precision (D values) decrease as the mean increases. Despite the fact that precision is improved with an increase in the sample sizes, gains in precision become minor at high sample sizes. Since, the level of the precision needed is a choice made based on the purpose of a sampling plan, according to facilities, capabilities and time, in the precision levels of 0.25 and 0.3, one hundred plants (500 tillers) were sampled from each (diagonal) line of the fields (Figure 1).

The results of RV and RNP analyses indicated that the best sample unit was 4 or 5 tillers per wheat plant (Table 1). According to RV analyses, there wasn't any significant difference between 4 and 5 tillers. Considering that the lower RV showed more precise and lower error, 4 stems was selected.

Distribution pattern

The distribution patterns of *S. avenae* on *T. aestivum* were established in accordance with Taylor's and Iwao's indices of dispersion. The result of the current study reveals the dispersion patterned of *S. avenae* to be highly aggregated within *T. aestivum* (Figures 2 and 3).

Taylor's power law analysis appeared to illustrate the distribution of *S. avenae* well by showing highly significant relationships between the variance and mean of *S. avenae* population (Figure 2). The slope values of Taylor's power law for this aphid was found to be significantly greater than 1 for Shiraz (t=8.12, df=133, P<0.0001) and bahar cultivars (t=8.5, df=126, P<0.0001), indicating an aggregated or clumped distribution pattern for *S. avenae* on *T. aestivum*. On the contrary, Iwao's patchiness regression based on the same sampled tillers did not show high significant relationship between the mean crowding index (m^*) and the mean (m) of *S. avenae* (Figure 3). Although, the constant α in the Iowa's model indicates the tendency to crowding when it is positive (+) or repulsion when it is negative (-) as it is the *index of basic contagion* defined by Iwao (1968).

Based on the higher value of R^2 made by Taylor's power law compared to Iwao's patchiness regression, it could be expressed that Taylor's model fitted the data better than Iwao's model. Furthermore, Taylor's power law provides a more even distribution of the points along the line than Iwao's model. In spite of Iwao's model inability to fit the data very well, it could still give an insight into the interpretation of implication of ecological parameters (Kuno, 1991). For instance, the positive value of α of Iwao's patchiness regression in the present study is indicative of a mutual attraction (positive interaction) between the individuals even at a low density.

The heterogeneity of slopes regression model indicated that neither the slope nor the intercept of Power Law regressions differed signifi-

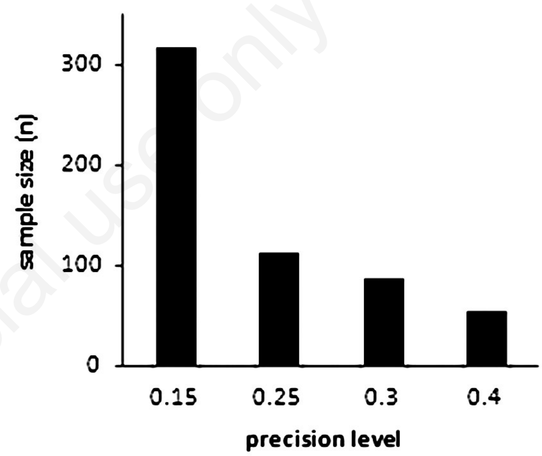

Figure 1. Sample sizes with different precision levels for *S. avenae* in wheat fields of Badjgah.

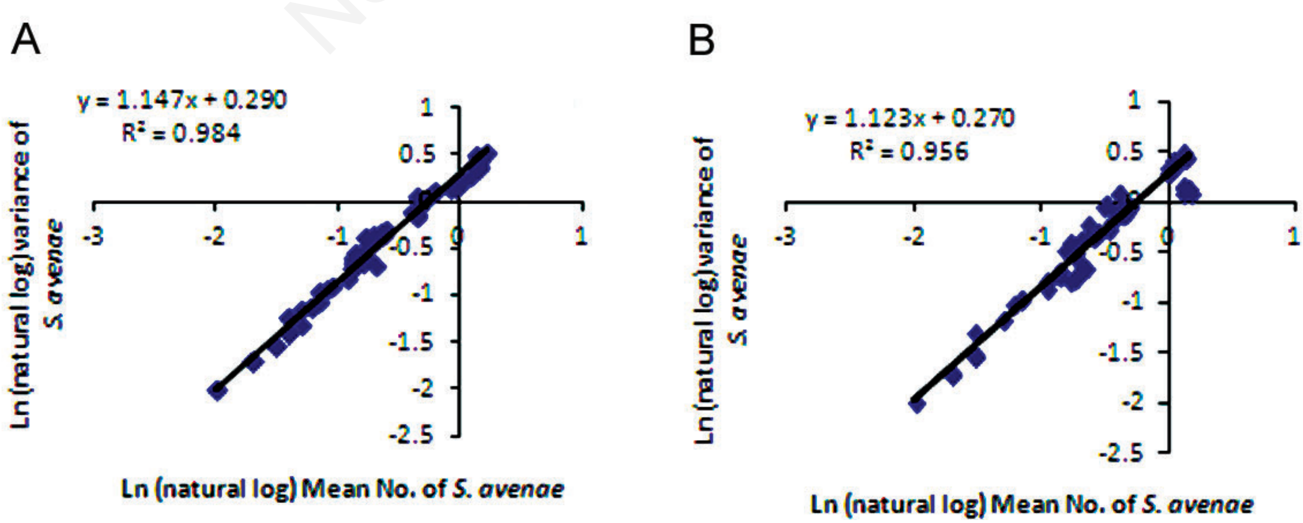

Figure 2. Regression analysis of Taylor's power law for *S. avenae* populations on *T. aestivum*; A) Shiraz cultivar, B) Bahar cultivar.

cantly for the two wheat cultivars (slope, df=99, t=1.06, intercept, df=99, t=1.28). In spite of this observation, Taylor's indices for two wheat cultivars were calculated together.

Constructing fixed percision sampling schemes

The relationship between the cumulative number of aphids and number of sample taken for the fixed precision levels of 0.25 and 0.30 and the stop lines for sequential sampling is showed in Figure 4. Since the variance mean regression in Taylor's model provided a good description of the data (Figure 2), the regression variability would only have a minor effect at very low mean density.

In order to achieve high fixed precision levels of 0.15 for precise number of sample taken, quite a large number of samples are required (Figure 4). For example in 15 sample plants (with four tillers) in D_{exp}=0.15, D_{exp}=0.25 and D_{exp}=0.3, 290, 118 and 46 aphids will be observed, respectively.

Validation of Green's model was evaluated using RVSP software. From the result of the present study, in precision level of 0.15, this program could not run. Resampling analysis for *S. avenae* with precision set at 0.25 resulted in an average sample number of 111 plants, ranging from 359 (0.06 aphids per sample unit) to 24 (1.46 aphids per sample unit). In precision level of 0.3, the average number of 78 samples ranged from 253 (0.07 aphids per sample unit) to 17 (1.48 aphids per sample unit) (Figure 5, Table 2).

Comparing number of samples in conventional methods with Green's method indicated that in precision levels of 0.25 and 0.3 the number of needed samples in Green's method compared to conventional one was reduced by 79.5 and 66 percent, respectively (Table 3).

Wilson and Room's model

Equations for the Wilson and Room model (based on *a* and *b* values calculated from Taylor's Power Law) are described by hyperbolic curves (Figure 5). According to the p-x relation, when 50% of the sampling units (4 stems) contain aphids, the mean number of aphids/ sampling unit is approx. 1 (Figure 6).

As can be seen based on Wilson and Room's model (1983), with the increase percentage of infected plants in the field, the number of required samples decreases to. For example, in *S. avenae*, when the proportion of infection was 0.5, in decision levels 0.15, 0.25 and 0.3 the sample's number was 143, 29 and 20, respectively (Figure 7).

Discussion and conclusions

Since evaluation of the spatial distribution pattern is a key element in pest management strategies, two methods of Taylor and Iwao were tested for *S. avenae* on *T. aestivum*. According to Hutchison *et al*. (1988), both of these two regression models can estimate insect population distribution parameters. In this research, Taylor's power law

Table 1. Results of relative variation and relative net precision analysis for *S. avenae* in wheat fields of Badjgah.

Analysis	Cultivar	1 stems	2 stems	3 stems	4 stems	5 stems
RV	Shiraz	55.69^{c}	$44b^{c}$	40.7^{c}	35.1^{d}	30.8^{d}
	Bahar	50.90^{a}	38.7^{b}	32.9^{c}	29.7^{d}	28.2^{d}
RNP	Shiraz	4.1^{a}	3.6^{b}	3.4^{bc}	3.2^{cd}	3^{d}
	Bahar	4.3^{a}	4^{a}	3.8^{c}	3.5^{cd}	3.3^{d}

[a,b,c,d]Means within a row followed by the same letter are not significantly different at the 5% confidence level according to Duncan's studentized range test. RV, relative variation; RNP, relative net precision.

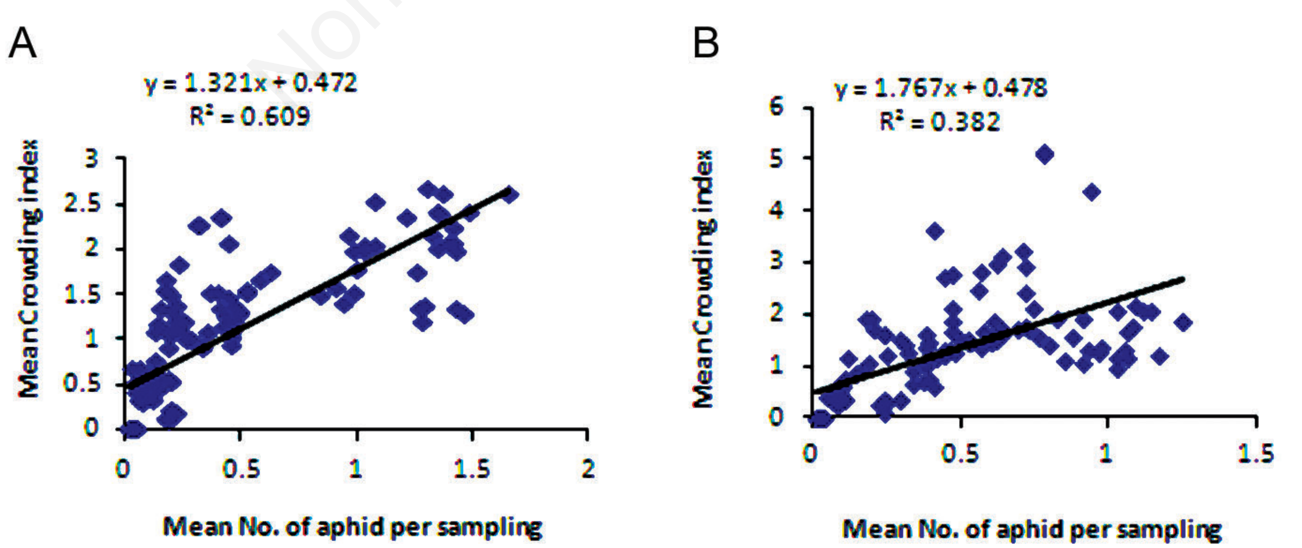

Figure 3. Regression analysis of Iwao's mean crowding index (m*) on mean density (m) for *S. avenae* populations on *T. aestivum*; A) Shiraz cultivar, B) Bahar cultivar.

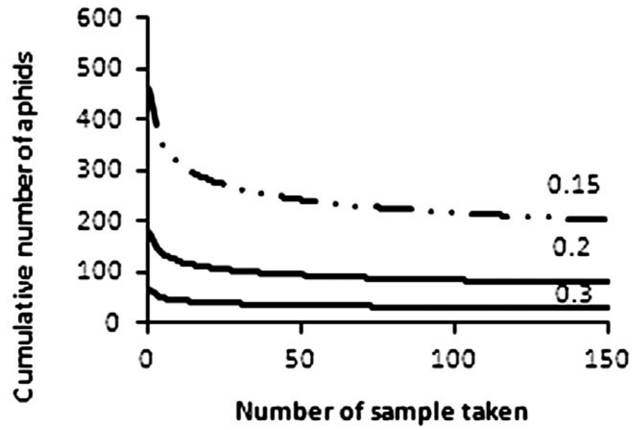

Figure 4. Sampling stop line at a fixed precision level of 0.15, 0.25 and 0.30 for *S. avenae* on *Triticum aestivum*.

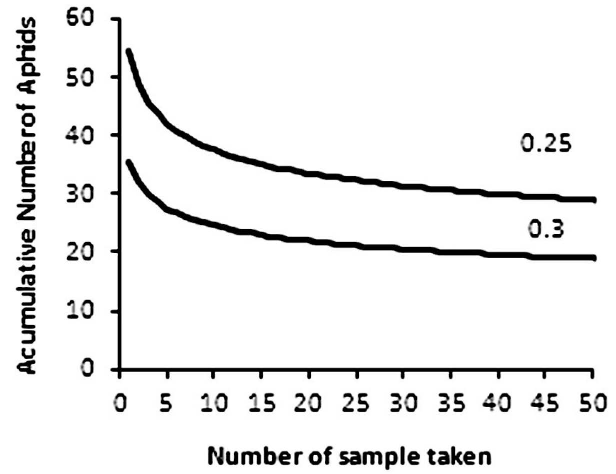

Figure 5. Summary of resembling validation analysis showing range of *S. avenae* densities over number of sample taken for Green's sequential sampling plan.

Table 2. Results of validation by resampling for validation of sampling plans software for D=0.25 and D=0.30 for *S. avenae* in wheat field of Badjgah.

Number of data	$Mean_{obs}$	Mean population		D (in simulation model) Mean		Higher		Lower		Number of sample Mean		Higher		Lower	
		0.25	0.03	0.25	0.03	0.25	0.03	0.25	0.03	0.25	0.03	0.25	0.03	0.25	0.03
1	0.06	0.06	0.07	0.27	0.32	0.30	0.37	0.24	0.27	359	253	670	623	140	105
2	0.09	0.10	0.10	0.24	0.29	0.28	0.33	0.20	0.25	250	177	418	338	118	75
3	0.11	0.12	0.12	0.23	0.27	0.27	0.33	0.20	0.22	210	147	344	310	109	66
4	0.18	0.20	0.21	0.32	0.38	0.40	0.51	0.23	0.24	141	10	320	253	49	31
5	0.20	0.23	0.24	0.32	0.38	0.41	0.48	0.21	0.26	127	91	251	209	48	21
6	0.26	0.27	0.28	0.26	0.31	0.31	0.36	0.21	0.24	105	73	193	168	42	28
7	0.38	0.42	0.43	0.28	0.33	0.34	0.44	0.20	0.21	72	52	137	108	33	20
8	0.44	0.47	0.48	0.25	0.30	0.34	0.41	0.19	0.21	65	45	106	91	32	19
9	0.49	0.54	0.53	0.24	0.29	0.03	0.37	0.17	0.20	57	42	96	83	29	20
10	0.58	0.63	0.63	0.26	0.31	0.35	0.43	0.20	0.21	51	37	93	72	26	15
11	0.93	0.97	0.97	0.20	0.24	0.29	0.38	0.13	0.15	34	24	57	38	21	13
12	01.03	01.10	01.13	0.25	0.30	0.32	0.41	0.16	0.17	31	22	59	44	15	9
13	01.14	01.18	01.23	0.24	0.28	0.31	0.39	0.17	0.18	29	20	49	45	14	10
14	01.42	01.46	01.48	0.17	0.20	0.24	0.28	0.10	0.10	24	17	35	26	16	10
Mean	0.52	0.55	0.55	0.25	0.29	0.31	0.38	0.18	0.20	111.7	78.56	202	171.99	49.4	31.57

Table 3. Number of samples of *S. avenae* using Green's method compared to conventional methods used in Badjgah.

Precision level	Conventional method Lower	Higher	Mean	Green Lower	Higher	Mean	Reduction of sample number Lower	Higher	Mean
0.25	73.1	6146	843	12	93	52	71.2	85.9	79.5
0.3	50.7	4268	585.4	9	83	46	0.55	88.4	66

analysis appeared to illustrate the distribution of *S. avenae* better by showing highly significant relationships between the variance and mean of *S. avenae* population.

This result corroborates the previous finding by Eliot & Kieckhefer (1987), who showed that Taylor's Power Law showed the spatial distribution of *S. avenae*, *Rhopalosiphum padi, R. maidis* and *Schizaphis graminum* better than Iwao. In addition, other results similar to ours were reported by previous studies (Dean & Luring, 1970; Feng & Nowierski, 1992; Burgio *et al*., 1995; Elliot & Kieckhefer, 1987; Athanassiou *et al*., 2005; Kavallieratos *et al*., 2002, 2005; Fievet *et al*., 2007; Tomanovic *et al*., 2008a; Afshari & Dastranj, 2010), on other aphid species, *S. avenae, R. padi, R. maidis* and *Schizaphis graminum*, *Metopolophium dirhodum, D. noxia* and *Myzus persicae*.

Many authors have reported that an aggregated distribution pattern is a predominant form of arthropod distribution and regular distribution is rare and mainly found in the population where there is a strong competition among individuals (Argov *et al*., 1999). The aggregated distribution pattern displayed by *S. avenae* in the present study might be attributed to food source, since *S. avenae* was reported to be more attracted to the ear and upper leaves of cereals for feeding (Gianoli, 2000) and, or to some variations of the environment such as microclimate and natural enemies (Gianoli, 2000; Tomanovic *et al*., 2008b; Elliott & Kieckhefer, 2000).

Sequential sampling models, due to its high accuracy, lower costs and faster decisions, have a special importance in the study of insect populations (Binns, 1994; Pedigo & Zeiss, 1996; Young & Young, 1998).

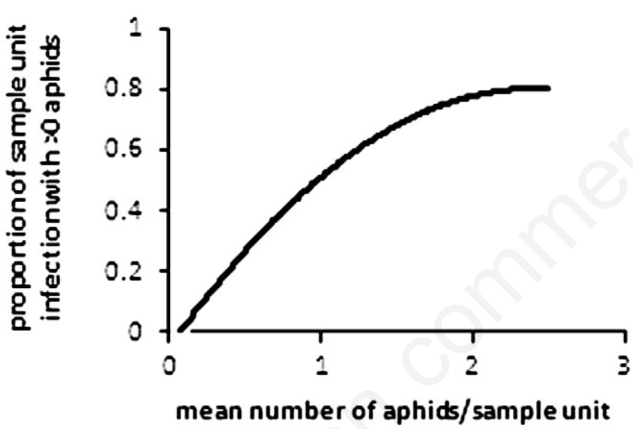

Figure 6. Relation between the proportion of sampling units (4 stems) that had one or more (*i.e.* >0) individuals of aphids, and the mean number of aphids per sample unit.

Comparing number of samples in conventional methods with Green's method indicated that in precision levels of 0.25 and 0.3, the number of needed samples in Green's method was reduced 79.5 and 66 percent, respectively compared to conventional one. This result is corroborated by the result of several authors (Mohiseni *et al.,* 2009; Afshari, 2009; Pieters & Sterling, 1975; Shahrokhi & Amir-Maafi, 2011).

Validation of Green's model was evaluated using RVSP software. Similar density-based, fixed- precision sequential sampling plans have been developed and validated using the resembling approach (Naranjo & Hutchison, 1997) for several insect species, including: *Macrosteles quadrilineatus* (O'Rourke *et al*., 1998), *Cryptolestes ferrugineus* (Subramanyam *et al*., 1997), *Acaymma vittatum* (Burkness & Hutchison, 1998), Leptinotarsa decemlineata (Hamilton *et al*., 1998), *Eurygaster integriceps* (Mohiseni *et al*., 2009) and *Schyzaphis graminum* (Afshari & Dastranj, 2010). Elliott *et al.* (2003) examined spatial distribution of *S. avenae* in South Dakota in 1993. They stated the number of samples 40-250 in precision D=0.25 (Elliott *et al*., 2003), while the results of this study showed 24-350 samples. This difference depends on the extent of the variation in relation to sampling scheme. This approach illustrates that, when adequate independent data set are used for validation, the final sequential sampling plans can be used with confidence to ensure that the desired fixed- precision levels are achieved.

In our study, Taylor's slope values showed an aggregated distribution pattern among sampling units. This aggregation of high numbers of individuals in a relatively low number of sampling units reduces the precision obtained in estimating mean insect density. Determining the proportion of leaves with >0 individuals can be considered as an alternative for estimating the mean number of aphid directly. Thus, if a specific threshold is established, based on the given mean density value, this mean can be predicted by simple presence/absence characterization of the samples, without counting the individuals found. Hence if this ratio can be accurately predicted from the p- $\overline{x}$ relation, insecticidal applications should be done when necessary (Wilson & Room, 1983). Our data suggest that Wilson and Room's model are useful and save time and cost. Based on this model, by increasing the percentage of infected plants in the field, the number of required samples reduced. This result is in accordance with results of Athanassiou *et al*. (2005) on *Myzus persicae* and *Macrolophus costalis.*

A sampling based management strategy in wheat is essential under the establishment of certain thresholds, which can vary among countries, pest species, plant varieties and so forth. The determination of these thresholds would encourage wheat farmers or managers to follow a sampling-based control strategy, under the principles of integrated pest

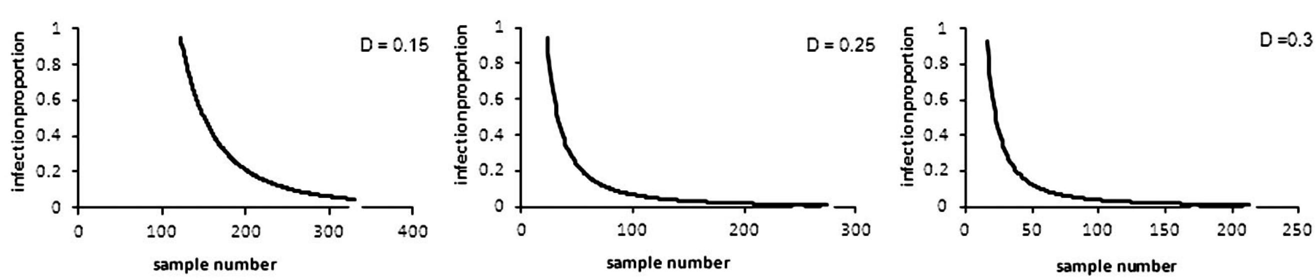

Figure 7. Number of samples required for estimating the population density of *S. avenae* in precision levels of 0.15, 0.25 and 0.3 in the fields of Badjgah based on Wilson and Room's model.

management. The findings of this study will go a long way in reducing the problem faced by farmers on decision-making with respect to pest.

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