Entomofauna	39/1	Heft 12: 285-300	Ansfelden, 2. Januar 2018

Fixed Precision Sequential Sampling of *Rhopalosiphum padi* (L.) in wheat fields of Badjgah (Fars Province) in Iran

Maryam ALEOSFOOR, Vajiheh SOLTANI GHASEMLOO & Amir MOHSENI

Abstract

The Bird cherry-oat aphid, *Rhopalosiphum padi* (L.), is a serious pest of cereals mainly due to its role as a vector of viruses. In this study, during 2013-2014 growing seasons, four hundred wheat plants were checked for *R. padi* on repeated occasions (twice a week). Based on the results, the aphid population was aggregated. The number of required samples in Green's method was reduced to 78.9 and 80.3 percent, respectively compared to the conventional one in precision levels of 0.25 and 0.3. Furthermore, information on spatial distribution and sequential sampling of *R. padi* provides an appropriate data base for selecting best decision making in designing IPM programs.

Key words: Iwao's regression model, population fluctuation, *Rhopalosiphum padi*, sequential sampling, spatial distribution, Taylor's power law.

Introduction

In Fars province (south of Iran), cereals has economic importance and over 463000 ha of agricultural lands are dedicated to the cultivation of winter wheat – *Triticum aestivum* L. (KHERAD 2015). The bird cherry-oat aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae) is regarded as one of the most important aphids of cereals in Fars province, Iran. This aphid, alternates between a winter host, bird cherry *Prunus padus* (Rosaceae), and summer hosts, a range of grasses (Gramineae) including cereals. It is an important pest of cereals, causing damage both as a virus vector and by direct feeding (HODJAT & AZMAYESHFARRD 1986). The preferred feeding site of *R. padi* (L.), on cereal seedlings is the stem base and lower leaves (DEAN 1973, LEATHER & LEHTI 1982).

The development of pivotal management strategies for agricultural pest needs, at least, a basic understanding of its dispersion and abundance for performance of control tactics that are least harmful to its natural enemies. Dispersion and abundance of organisms are the most important features 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 connection that exists between organism and its environment which may be useful in scheduling efficient sampling programs for population estimates, development of population models and pest management strategies (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).

Fixed precision sequential sampling schemes are based on mathematical models, such as Taylor's power law (TAYLOR 1961), which in this case represents the sample variance as a function of the mean number of aphids per sample 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 efforts, cost and minimize variation of sampling precision (PAYANDEH et al. 2010).

Sequential sampling is one of the useful ways that can play a main role in the implementation of integrated pest management (IPM) strategies (BINNS 1994), and plans have been developed for pests in many crop systems (PEDIGO & BUNTIN 1994).

Despite the fact that Fars province has the first rank of wheat production in Iran (KHERAD 2015), and economic importance of *R. padi* to wheat growers, until recently, few sequential sampling plans have been available for this aphid.

Indispensable to achieving this goal, for obtaining information on the distribution and abundance of the aphid, this study was undertaken to determine dispersion pattern of *R.padi* in order to develop a suitable sampling plan for this pest.

Material and methods

Study site and population sampling

The population fluctuation of *R. padi* was studied in wheat fields at two pesticide- free rectangular wheat fields at Badjgah region, Fars province (N 52 42´ E 29 50´) from late-March to late-July in 2013- 2014. Each field has an area of 2 hectares. Two cultivars, Shiraz and Bahar, were planted separately and agronomic practices, such as application of manure, were given to wheat fields at regular intervals.

Aphids were counted 2 days per week throughout the growing season (from initiation of tillering till grain ripening stage, each week 400 samples were counted In Situ), unless rainfall increased intervals between sampling dates. Tillers were collected by traveling an X- shaped procedure and the data from primary sampling were then used to develop sample size for the the bird cherry-oat aphid using formula (1) described by KARANDINOS (1976).

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

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 suitable sample unit was estimated by calculating the relative variation (RV) using formula (2).

$$RV = \frac{SE}{mean} * 100 \qquad (2)$$

Where RV and SE, are the relative variation and Standard error of mean (PEDIGO et al. 1972, KARANDINOS 1976, ZAR 2010, HALL et al. 1991, BUNTIN 1994).

Taylor's power law (TPL)

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

$$Log(S^2) = Log(\alpha) + bLog(\bar{x}) \qquad (3)$$

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. When 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 (IWAO 1968):

$$m^* = \alpha + \beta m \qquad (4)$$

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:

$$t = (slope - 1) / SE_{slope} \qquad (5)$$

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 degree 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 (6) with (N_1+N_2)-2 degrees of freedom (FENG & NOWIERSKI 1992).

$$t_{slope} = (b_1 - b_2) / \sqrt{(SE_{b_1}{}^2 + SE_{b_2}{}^2)} \qquad (6)$$

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 Low 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.

Green's method, minimum sample sizes needed to achieve a fixed level of precision (*Dexp*), depend on SE/$\bar{x}$ calculated from Green's (1970) formula:

$$n = \frac{\alpha \bar{x}^{b-2}}{D_{\exp}^{2}} \quad (7)$$

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:

$$T_n = \left(\frac{D_{\exp}^{2}}{\alpha}\right)^{1/(b-2)} n^{(b-1)/(b-2)} \quad (8)$$

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 Taylor' Power Law. Validation of Green's model was evaluated using RVSP software. Then, the numbers of samples in conventional method and Green's method were compared (SHAHROKHI & AMIR-MAAFI 2011, MOHISENI et al. 2008).

Wilson and Room's model

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

$$P(I) = 1 - e^{-\bar{x} In(a.\bar{x}^{b-1})(a.\bar{x}^{b-1}-1)^{-1}} \quad (9)$$

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 ($\bar{x}$) from a simple count of the proportion of sampling units in which this species is present (*p*).

Results

Seasonal Activity of R. padi in wheat fields

Population fluctuation curves of *R. padi* are shown in Figure 1. The population of *R. padi* was observed from the beginning of the sampling period (1 April). It increased rapidly during subsequent weeks. The population peak of aphid, which occurred in the 2nd half of April, reached 16.52 and 16 individuals per sample unit in 2013 and 2014, respectively. Furthermore, the severe decline in the *R. padi* population from late April in two years could mainly be due to the predator's action, plant-pest interaction or weather conditions after which the population density of the pest fluctuates irregularly (Fig. 1). It seemed that aphids' population was adversely affected by average temperature but humidity had a consonant effect on it.

Spatial Distribution and Sample Size Determination

Required sample sizes increased with decreased aphid populations and increased levels of precision. 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, 500 tillers of one hundred plants were taken from each (diagonal) line of the fields.

According to calculated RV, there wasn't any significant difference between 4 and 5 tillers. Considering that the lower RV showed more precise and lower error, 4 stems were selected (Tab. 1).

Taylor's power law and Iwao's patchiness regression were used to analyze the spatial distribution of the aphids. *Rhopalosiphum padi* on *T. aestivum* exhibited an aggregated distribution. Based on higher value of R^2, Taylor's power law provided a better fit to the data than Iwao's patchiness regression (Figs. 2- 3).

The slope values of Taylor's power law for this aphid was found to be significantly greater than 1 for Shiraz ($t = 9.16$, df = 72, $p < 0.0001$) and Bahar cultivars (t=20.5, df = 74, $p < 0.0001$), indicating an aggregated or clumped distribution pattern for *R.padi* on *T. aestivum*. On the contrary, Iwao's patchiness regression based on the same sampled tillers did not show A high significant relationship between the mean crowding index (*m*) and the mean (*m*) of *R.padi* (Fig. 3). Although 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 (df= 99, t=0.75) nor the intercept (df= 99, t=3.56) of Power Law regressions differed significantly for the two wheat cultivars, so data from both cultivars were pooled and Taylor's indices were calculated.

Constructing fixed percision sampling schemes

Sequential sampling stop lines for three levels of desired sampling precision (0.15, 0.25 and 0.3) are provided for Green's method in Figure 4. Since the variance mean regression in Taylor's model provided a good description of the data (Fig. 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 (Fig. 4). For example in 50 sample plants (with four tillers) in $D_{exp} = 0.15$, $D_{exp} = 0.25$ and $D_{exp} = 0.3$, 1100, 480 and 220 aphids will be observed, respectively.

Validation of Green's model

Using Green's method, the resampling for validation of sampling plans (RVSP) program was used to validate the sequential sampling plans of *R.padi* (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 *R.padi* 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).

RVSP software could not run the program at precision level of 0.15. Resampling analysis for *R.padi* with precision set at 0.25 resulted in an average sample number of 108 plants, ranging from 461 (0.09 aphids per sample unit) to 16 (12.46 aphids per sample unit). In precision level of 0.3, the average number of 62 samples ranged from 260 (0.09 aphids per sample unit) to 12 (12.27 aphids per sample unit) (Fig. 5, Tab 2).

One of the advantages of Green's method is reducing the number of required samples compared to the conventional one. Based on the results, in precision levels of 0.25 and 0.3 the number of required samples in Green's method was reduced TO 78.9 and 80.3 percent, respectively compared to the conventional one (Tab 3).

Wilson and Room's model

Wilson and Room's model, based on the Taylor's estimates, was used to calculate the mean number of aphids, from the proportion of sampling units that had > 0 individuals of this species, described by hyperbolic curves (Fig. 6). Equations are given for the calculation of precision in estimating the mean number of aphids per sampling unit, and the required sample size for a given level of precision. 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 (Fig. 6).

According to Fig. 7, with the increase percentage of infected plants in the field, the number of required samples decreases. For example, when the proportion of infection was 0.5, in decision levels 0.15, 0.25 and 0.3, the sample's number was 95, 35 and 15, respectively.

Discussion

According to the results of our study (Fig. 1), under the conditions prevailing in the study area, aphid densities were established in wheat fields during April, approximately when tillers have formed. Late in April, at the end of Stem elongation, *R. padi* densities decreased notably, probably as the result of high temperature and low humidity.

AHEER et al. (1994 and 2007), JAROSIK et al. (2003) reported that high temperature had a negative role on aphid population, as humidity played a positive role. These findings are in partial agreement with our results.

For distribution analyses two models of Taylor and Iwao were used for *R.padi* on *T. aestivum*. In this research, Taylor's power law analysis appeared to illustrate the distribution of *R.padi* better by showing highly significant relationships between the variance and mean of *R.padi* population. These results are similar to those of DEAN & LURING 1970, ELIOT & KIECKHEFER 1987, FENG & NOWIERSKI 1992, BURGIO et al. 1995, ATHANASSIOU et al. 2005, KAVALLIERATOS et al. 2002, 2005, FIEVET et al. 2007, TOMANOVIC et al. 2008a, AFSHARI & DASTRANJ 2010 and SOLTANI GHASEMLOO & ALEOSFOOR 2014. These authors found that Taylor's power law provides a more even distribution of the points along the line than Iwao's model. It fitted the data better than Iwoa's regression modal on other aphid species, *S. avenae*, *R. padi*, *R. maidis* and *Schizaphis graminum*, *Metopolophium dirhodum*, *D. noxia* and *Myzus persicae*. In spite of Iwao's model inability to fit the data very well, it could still give an understanding of the explanation of concept of ecological parameters (KUNO 1991).

The slope values of Taylor's power law for the *R.padi* on wheat was significantly greater than 1, indicating an aggregated or clumped distribution pattern. Many authors have reported that an aggregated distribution pattern is a prevailing 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 illustrated by *R.padi* in the present study might be due to food source, since the preferred feeding site of the bird cherry-oat aphid on cereal seedlings is the stem base and lower leaves (DEAN 1974, LEATHER & LEHTI 1982) and, or to some variations of the environment such as climate changes and natural enemies (GIANOLI 2000, TOMANOVIC et al. 2008b, ELLIOTT & KIECKHEFER 2000).

The fixed precision sampling models developed in this study will provide useful insights about efficient estimation of *R. padi* population density in a cost-effective manner. Furthermore, information on the density level of *R. padi* provides a faster and higher accuracy decision for selecting an appropriate method in IPM programs (BINNS 1994, PEDIGO & ZEISS 1996, YOUNG & YOUNG 1998).

Reduction in the number of needed samples is one of the advantages of the Green's method compared to the conventional one. Based on the results, in precision levels of 0.25 and 0.3, the number of needed samples in Green's method was reduced to 78.9 and 80.3 percent, respectively compared to conventional one. This result corroborates the previous finding by MOHISENI et al. 2008, AFSHARI 2009, PIETERS & STERLING 1975, SHAHROKHI & AMIR-MAAFI 2011 and SOLTANI GHASEMLOO & ALEOSFOOR 2014).

Reliable and cost-effective sampling methods are necessary to establish the proper monitoring systems in pest management. Sampling plans are often developed from a restricted range of observations from a small area, but are then used over a wide area representing a novel array of environmental and agronomic conditions. By using RVSP software, actual field data is resampled to evaluate sample plan performance (NARANJO & HUTCHISON 1997).

Similar sequential sampling plans have been validated using the resembling approach until the stop line had been reached (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. 2008), *Schyzaphis graminum* (AFSHARI & DASTRANJ 2010) and *Sitobion avenae* (SOLTANI GHASEMLOO & ALEOSFOOR 2014). This shows that when sufficient independent data sets are used for validation, the final sequential plans can be used with confidence.

In our study, Taylor's power law parameters from the regression of log variance versus log mean suggested an aggregated distribution. This aggregation of high numbers of aphids in a relatively low number of sample units decreases the precision achieved in estimating mean insect density. Defining the proportion of plants with >0 individuals can be considered as another suggestion for estimating the mean number of aphids. Therefore, based on the given mean density value, a specific threshold will be determinated. This mean can be predicted by simple presence/ absence of aphids, without counting the individuals. So if this ratio can be correctly forecast from the p-$\bar{x}$ relation, control programs should be done when required (WILSON & ROOM 1983). Saving time and cost is one of the advantages of Wilson and Room's model. Based on this model, by increasing the percentage of infected plants in the field, the number of required samples decreased. Similar results can be seen in ATHANASSIOU et al. (2005) that is in accordance with our results.

The fixed-precision sequential sampling plans for *R. padi* will be useful for rapid estimation of aphid densities in wheat for large-scale research programs designed to assess the best control strategies (ELLIOTT et al. 2003). Furthermore, information on the spatial distribution and sequential sampling of *R. padi* provides a data base for selecting appropriate decision making in designing IPM programs for this particular pest.

Acknowledgment

This research was supported by Shiraz University research grant, and the authors are grateful to the Agricultural College of Shiraz University for providing the wheat fields used for this study. This manuscript benefited greatly from comments by Dr. Lida Fekrat. The authors also thank Dr. M. S. Bagheri for professional English-language editing of the manuscript.

References

AFSHARI A., SOLEIMAN NEGADIAN E. & P. SHISHEBOR (2009): Population Density and Spatial Distribution of *Aphis gossypii* GLOVER (Homoptera: Aphididae) on Cotton in Gorgan. – Iran-Journal of Agricultural Science and Technology **11**: 27-38.

AFSHARI A. & M. DASTRANJ (2010): Density, Spatial distribution and sequential sampling plans for cereal aphids infesting wheat spike in Gorgan, northern Iran - Plant Protection. Scientific. – Journal of Agriculture **32**: 89-102.

AHEER G.M., AHMAD K.J. & A. ALI (1994): Role of weather in fluctuating aphid density in wheat crop. – Journal of Agricultural Research **32** (3): 295-301.

AHEER G.M., MUNIR M. & A. ALI (2007): Impact of weather factors on population of wheat aphids at Mandi BAHA-UD-DIN District. – Journal of Agricultural Research. **45** (1): 61-66.

ARGOV Y., ROSSLER Y., VOET H. & D. ROSE (1999): Spatial Dispersion and Sampling of Citrus Whitefly, *Dialeurodes citri*, for Control Decisions in Citrus Orchards. – Agricultural and Forest Entomology **1**: 305-318.

ARNALDO P.S. & L.M. TORRES (2005): Spatial Distribution and Sampling of *Thaumetopoea pityocampa* (DEN. & SCHIFF.) (Lepidoptera: Thaumetopoeidea) Populations on *Pinus pinaster* Ait. in Montesinho, N. Portugal. – Forest Ecology and Management **210**: 1-7.

ATHANASSIOU C.G., KAVALLIERATOS N.G., TOMANOVIC Z., TOMANOVIC S. & M. MLUTINOVIC (2005): Development of a sampling plan for *Myzus persicae* (Sulzer) (Hemiptera: Aphidoidea) and its predator *Macrolophus costalis* Fieber (Hemiptera: Miridae) on tobacco. – European Journal of Entomology **102**: 399-405.

BINNS M.R (1994): Sequential Sampling for classifying pest status. – In: PEDIGO L.P. & G.D. BUNTIN (eds), Handbook of Sampling Methods for Arthropods in Agriculture: 137-174, CRC Press.

BISSELEUA D.H.B., YEDE P. & S. VIDAL (2011): Dispersion Models and Sampling of Cacao Mirid Bug *Sahlbergella singularis* (Hemiptera: Miridae) on *Theobroma cacao* in Southern Cameroon. –Environmental Entomology **40** (1): 111-119.

BUNTIN G.D. (1994): Developing a primary sampling program. – In: PEDIGO L.P. & G.D. BUNTIN (eds.), Handbook of Sampling Methods for Arthropods in Agriculture: 99-118, CRC Press.

BURGIO G., CORNALE R., CAVAZZUTI C. & M. POZZATI (1995): Spatial distribution and binomial sampling of *Sitobion avenae* and *Rhopalosiphum padi* L. (Homoptera: Aphididae) infesting wheat in northern Italy. – Bollettino dell'Istituto di Entomologia della universita di Bologna **50**: 15-27.

BURKNESS E.C. & W.D. HUTCHISON (1998): Development and validation of a fixed-precision sampling plan for estimating striped cucumber beetle (Coleoptera: Chrysomelidae) density in cucurbits. – Environmental Entomology **27**: 178-183.

DAVIS P.M. (1994): Statistics for describing populations. – In: PEDIGO L.P. & G.D. BUNTIN (eds), Handbook of Sampling Methods for Arthropods in Agriculture: 33-54, CRC Press.

DEAN G.J. (1973): Distribution of aphids in spring cereals. – Journal of Applied Ecology **10**: 447-462.

DEAN G.J. (1974): The four dimensions of cereal aphids – Annals of Applied Biology **77**: 74-78.

DEAN G.J. & B.B. LURING (1970): Distribution of aphid in cereal crops. – Annals of Applied Biology **66**: 485-496.

ELLIOTT N.C. & R.W. KIECKHEFER (1987): Spatial distributions of cereal aphids (Homoptera: Aphididae) in winter wheat and spring oats in South Dakota. – Environmental Entomology **16**: 896-901.

ELLIOTT N.C. & R.W. KIECKHEFER (2000): Response by coccinellids to spatial variation in cereal aphid density. – Population Ecology **42**: 81-91.

ELLIOTT N.C, GILES K.L., ROYER T.A., KINDLER S.D., TAO F.L., JONES D.B. & G.W. CUPERUS (2003): Fixed precision sequential sampling plans for the Green bug and bird cherry-oat aphid (homoptera:aphididae) in winter wheat. – Journal of Economic Entomology **96**: 1585-1593.

FAUVERGUE X. & K.R. HOPPER (1994): Spatial Distribution of *Diuraphis noxia* and one of its Parasitoids, *Diareretiella rapae*. – Proceedings of the Sixth Russian Wheat Aphids Workshop-Fort Collins, Colorado: 23-25.

FENG M.G. & R.M. NOWIERSKI (1992): Spatial distribution and sampling plans for four species of cereal aphids (Homoptera: Aphididae) infesting spring wheat in southwestern Idaho. – Journal of Economic Entomology **85**: 830-837.

FIEVET V., DEDRYVER C.A., PLANTAGENEST M., SIMON J.C. & Y. OUTREMAN (2007): Aphid colony turn-over influences the spatial distribution of the spatial distribution of the grain aphid *Sitobion avenae* over the wheat growing season- Agric. – Agricultural and Forest Entomology **9**: 125-134.

GIANOLI E. (2000): Competition in Cereal Aphids (Homoptera: Aphididae) on Wheat Plants. – Environmental Entomology **29** (2): 213-219.

GREEN R.H. (1970): On fixed precision level sequential sampling. Researches on Population. – Ecology **12**: 249-251.

HALL D.G., CHILDERS C.C. & J.E. EGER (1991): Estimating citrus rust mite (Acari: Eriophydidae) levels on fruit in individual citrus trees. – Environmental Entomology **20**: 382-390.

HAMILTON G.C., LASHOMB J.H., ARPAIA S., CHIANESE R. & M. MAYER (1998): Sequential sampling plans for Colorado potato beetle (Coleoptera: Chrysomelidae) in eggplant. – Environmental Entomology **27**: 33-38.

HODJAT S.H. & P. AZMAYESH FARD (1986): Aphids of wheat and other graminaceous of Iran. – Plant Pests and Disease **54** (1-2): 83-109.

IWAO'S (1968): A new regression method for analyzing the aggregation pattern of animal populations. – Researches on Population Ecology **10**: 1-20.

JAROSIK V., HONEK A. & A. TICHOPAD (2003): Comparison of field population growths of three cereal aphid species on winter wheat. – Plant Protection Science **39**: 61-64.

KARANDINOS M.G. (1976): Optimum sample size and comments on some published formulae. – Bulletin of the Entomological Society of America **22**: 417-421.

KAVALLIERATOS N.G., ATHANASSIOU C.G., STATHAS G.J. & Z. TOMANOVIC (2002): Aphid parasitoids (Hymenoptera: Braconidae: Aphidinae) on citrus: seasonal abundance, association with the species of host plant and sampling indices. – Phytoparasitica **30**: 365-377.

KAVALLIERATOS N.G., ATHANASSIOU C.G., TOMANOVIC Z., SCIARETTA A., TREMATERRA P. & V. ZINK (2005): Seasonal occurrence, spatio-temporal distribution and sampling indices for *Myzus persicea* (SULZER) (Hemiptera: Aphidoidea) and its parasitoids (Hymenoptera: Braconidae: Aphidiinae) on tobacco. – European Journal of Entomology **102**: 459-468.

KHERAD M. (2015): Fars has the first rank in wheat production. Available from: http://www.fars.agri-jahad.ir.

KUNO E. (1991): Sampling and analysis of insect populations. – Annual Review of Entomology **36**: 285-304

LEATHER S.R. & J.P. LEHTI (1982): Field studies on the factors affecting population dynamics of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) in Finland. – Annales Agriculturae Fenniae **21**: 20-31.

MOHISENI A.A., SOLEYMAN NEJADIAN E., RAJABI G.H. & M.S. MOSSADEGH (2008): Sequential sampling of overwintered sunn pest, Eurygaster integriceps (Het.: Scutelleridae) in rainfed wheat fields in Borujerd. – Iranian Journal of Agricultural Sciences **32**: 33-47.

NARANJO S.E. & W.D. HATCHISON (1997): Validation of arthropod sampling plans using a resampling approach: Software and analysis. – American Entomologist **43**: 48-47.

O'ROURKE P.K., BURKNESS E.C. & W.D. HUTCHISON (1998): Development and validation of a fixed-precision sequential sampling plan for aster leafhopper (Homoptera: Cicadellidae) in carrot. – Environmental Entomology **27**: 1463-1468.

O'ROURKE P.K. & W.D. HUTCHISON (2003): Sequential sampling plans for estimating European corn borer. (Lepidoptera: Crambidae) and corn earworm (Lepidoptera: Noctuidae) larval density in sweet corn ears. – Journal of Crop Protection **22**: 903-909.

PAYANDEH A., KAMALI K. & Y. FATHIPOUR (2010): Population structure and seasonal activity of *Ommatissus lybicus* in Bam Region of Iran (Homoptera: tropiduchidae). – Munis Entomology & Zoology **5**: 726-733.

PEDIGO L.P., LENTZ G.L., STONE T.D. & D.F. COX (1972): Green clover worm populations in Iowa soybean with special reference to sampling procedures. – Journal of Economic Entomology **65**: 414-421.

PEDIGO L.P. & G.D. BUNTIN (1994): Handbook of sampling methods for arthropods in agriculture. – CRC Press: 96p.

PEDIGO L.P. & M.R. ZEISS (1996): Analyses in Insect Ecology and Management. – Iowa State University Press. Ames: 168 pp.

PIETERS E.P. & W.L. STERLING (1975): Sequential sampling cotton squares damaged by boll weevils or *Heliothis* spp. In the costal bend of Texas. – Journal of Economic Entomology **68**: 543-545.

SAS (1999): User's Guide: Statistics. Version 9. SAS Institute Inc. Cary. NC. USA.

SHAHROKHI S.H. & M. AMIR-MAAFI (2011): Binomial sampling plan of *Metopolophium dirhodum* in irrigated wheat fields. – Applied Entomology and Phytopathology **79**: 117-133.

SISWANTO MUHAMAD R., OMAR D. & E. KARMAWATI (2008): Dispersion Pattarn of *Helopeltis antonii* SIGNORET (Hemiptera: Miridae) on Cashew Plantation. – Indonesian Journal of Agricultural Sciences **1** (2): 103-108.

SOEMARGONO A., IBRAHIM Y., IBRAHIM R. & M.S. OSMAN (2008): Spatial Distribution of the Asian Citrus Psyllid, *Diaphorina citri* KUWAYAMA (Homoptera: Psyllidae) on Citrus and Orange Jasmine. – Journal of Biosciences **19** (2): 9-19.

SOLTANI GHASEMLOO V. & M. ALEOSFOOR (2014): Dispersion pattern and fixed precision sequential sampling of *Sitobion avenae* (Fabricus) (Hemiptera: Aphididae) in wheat fields of Badjgah (Fars province) in Iran. – Journal of Entomological and Acarological Research **45**: 120-127.

SOUTHWOOD T.R.E. (1978): Ecological Methods with Particular Reference to the Study of Insect Populations. – John Wiley and Sons. New York: 524 pp.

SUBRAMANYAM B.H., HAGSTROM D.W., MEAGHER R.L., BURKNESS E.C., HUTCHISON W.D. & S.E. NARANJO (1997): Development and evaluation of sequential sampling plans for *Cryptolestes ferrugineus* (STEPHENS) (Coleoptera: Cucujidae) infesting farm-stored wheat. – Journal of Stored Products Research **33**: 321-329.

SULE H., MUHAMAD R., OMAR D., HEE A.K.W. & C. ZAZALI (2012): Dispersion Pattern and Sampling of *Diaphorina citri* KUWAYAMA (Hemiptera:Psylidae) Populations on *Citrus suhuiensis* HORT. Ex TANAKA in Padang Ipoh Terengganu, Malaysia. – Pertanika Journal of Tropical Agricultural Science **35**: 25-36.

TAYLOR L.R. (1961): Aggregation, variance and the mean. – Nature **189**: 732-735.

TAYLOR L.R. (1984): Assessing and interpreting the spatial distributions of insect populations. – Annual Review of Entomology **29**: 321-357.

TOMANOVIC Z., KAVALLIERATOS N.G. & C.G. ATHANASSIOUS (2008a): Spatial distribution of cereal aphids (Hemiptera: Aphidoidea) in Serbia. – Acta entomologica Serbica **13**: 9-14.

TOMANOVIC Z., KAVALLIERATOS N.G., STAR P., PETROVIĆ-OBRADOVIĆ O., ATHANASSIOU C.G. & L. STANISAVLJEVIĆ (2008b): Cereal aphids (Hemiptera: Aphidoidea) in Serbia: Seasonal dynamics and natural enemies. – European Journal of Entomology **105**: 495-501

WILSON L.T. & P.M. ROOM (1983): Clumping patterns of fruit and arthropods in cotton, with implications for binomial sampling. – Environmental Entomology **12**: 50-54.

YOUNG J.L. & J.H. YOUNG (1998): Statistical Ecology. – Kluwer Academic Publishers. Boston: 565 pp.

ZAR J.H. (2010): Biostatistical analysis. – fifth edition. Prentice-Hall/ Pearson: 944.

Authors' addresses:

Maryam ALEOSFOOR (corresponding Author)
Associate Prof., Department of Plant Protection,
College of Agriculture, Shiraz University, Shiraz, Iran
E-mail: aosfoor@shirazu.ac.ir

Vajiheh SOLTANI GHASEMLOO
Post graduate student, Department of Plant Protection,
College of Agriculture, Shiraz University, Shiraz, Iran
E-mail: v.soltani84@yahoo.com

Amir MOHSENI
Assistant Prof., Agricultural and Natural Resources of Lorestan-
Borujerd Agricultural Research Statioin, Borujerd, Iran
E-mail: mohiseni@yahoo.com

Tab. 1. Results of calculated RV for *R.padi* in wheat fields of Badjgah.

Analysis	Cultivar	1 stems	2 stems	3 stems	4 stems	5 stems
RV*	Shiraz	[a]48	[a]48.9	[a]44.7	[a] 43.7	[a] 42
	Bahar	[a]38.1	[a]32.9	[b] 29.3	[b] 28.7	[b]28.6

Means within a row followed by the same letter are not significantly different at the 5% confidence level according to Dancan's studentized range test.

*RV: Relative variation

Tab. 2. Results of validation by RVSP software for D = 0.25 and D=0.3 for *R.padi* in wheat field of Badjgah.

Number of data	$mean_{obs}$	Mean population		D) in simulation model						Number of sample					
				Mean		Higher		Lower		Mean		Higher		Lower	
		0.25	0.3	0.25	0.3	0.25	0.3	0.25	0.3	0.25	0.3	0.25	0.3	0.25	0.3
1	0.08	0.09	0.09	0.27	0.36	0.31	0.43	0.21	0.26	461	260	872	479	200	106
2	0.14	0.14	0.14	0.26	0.34	0.30	0.43	0.20	0.24	328	178	579	354	174	80
3	0.22	0.22	0.24	0.26	0.34	0.30	0.41	0.22	0.26	227	124	361	313	124	62
4	0.32	0.34	0.36	0.28	0.37	0.36	0.53	0.17	0.22	166	95	273	176	85	30
5	0.41	0.44	0.45	0.28	0.37	0.35	0.50	0.19	0.22	139	80	256	145	61	32
6	1.51	1.58	1.68	0.27	0.36	0.37	0.55	0.19	0.23	54	30	99	65	31	13
7	4.95	0.25	5.13	0.25	032	0.42	0.64	0.15	0.17	22	14	37	25	16	12
8	5.60	5.91	6.02	0.32	0.39	0.48	0.68	0.17	0.17	21	13	35	25	16	12
9	6.47	6.72	6.40	0.22	0.27	0.34	0.53	0.11	0.11	19	12	30	18	16	12
10	7.33	7.38	7.33	0.23	0.28	0.35	0.58	0.13	0.14	18	12	29	18	16	12
11	9.67	9/59	9.76	9.59	0.22	0.31	0.40	0.17	0.11	16	12	21	15	16	12
12	10.17	10.02	10.17	0.23	0.26	0.47	0.52	0.09	0.10	16	12	23	15	16	12
13	11.16	10.89	11.05	0.21	0.24	1.37	0.45	0.10	0.10	16	12	19	12	16	12
14	12.35	12.46	12.27	0.20	0.22	0.36	0.44	0.10	0.11	16	12	19	12	16	12
Mean	**5.02**	**5.06**	**5.07**	**0.24**	**0.31**	**0.43**	**0.50**	**0.15**	**0.17**	**108.5**	**61.85**	**189.5**	**119.42**	**57.35**	**29.92**

Tab. 3. Number of samples of *R.padi* using Green's method compared to conventional methods used in Badjgah

Precision level	Conventional method			Green			Reduction of sample number		
	lower	higher	mean	lower	higher	mean	lower	higher	mean
0.25	25.5	188	75.5±5.4	6	23	78.9±1.07	63.9	91.4	78.9±1.07
130.5	17.7	0.3	52.4±3.7	4	13	80.3±0.93	62.8	91.6	80.3±0.93

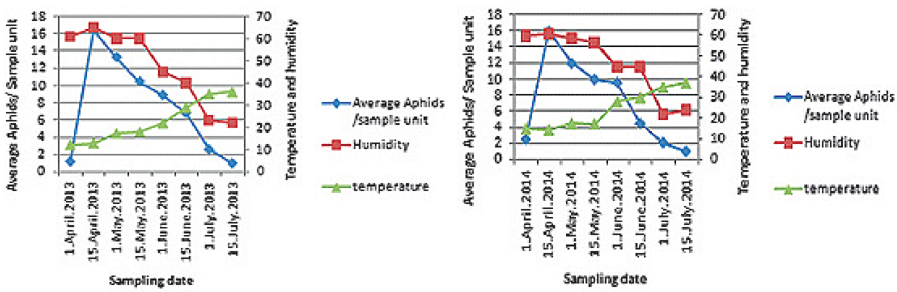

Fig. 1. Population fluctuation of *R. padi* in fields of Badjgah in 2013 and 2014.

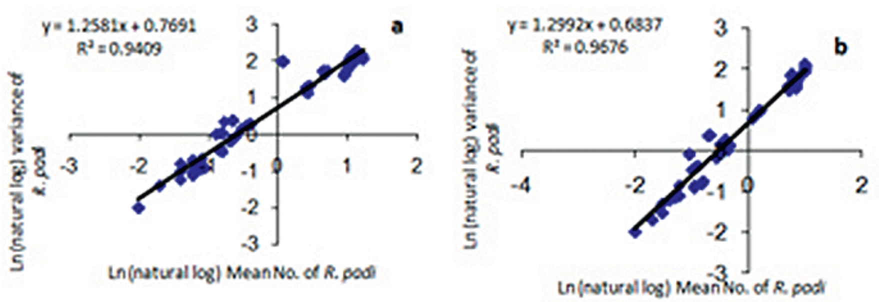

Fig. 2. Regression analysis of Taylor's power law for *R.padi* populations on *T. aestivum*.

a: Shiraz cultivar **b**: Bahar cultivar

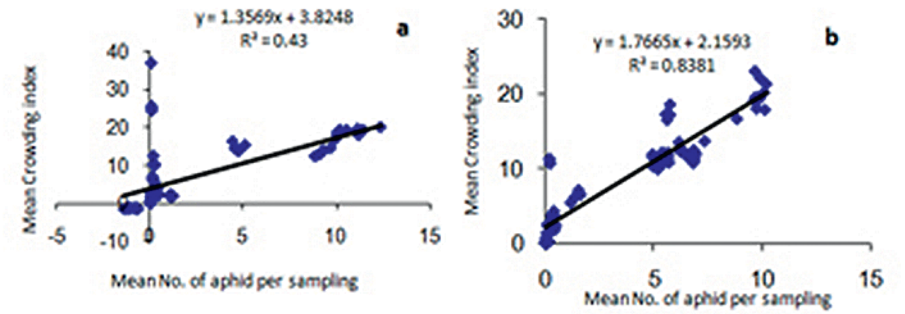

Fig. 3. Regression analysis of Iwao's mean crowding index (m*) on mean density (m) for *R.padi* populations on *T. aestivum*.

a: Shiraz cultivar **b**: Bahar cultivar

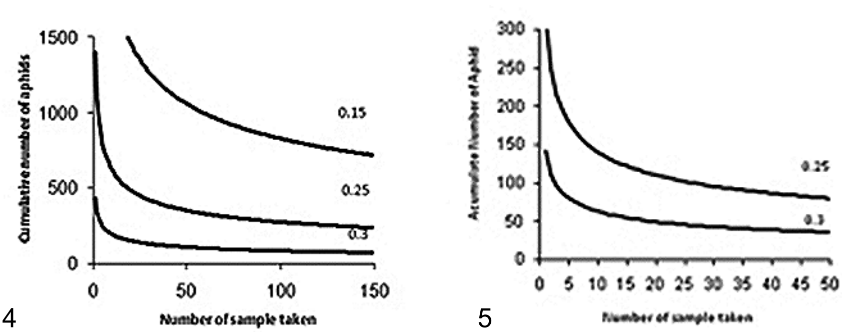

Fig. 4-5. (**4**) Sampling stop line at a fixed precision level of 0.15, 0.25 and 0.30 for *R.padi* on *Triticum aestivum*. (**5**) Summary of resembling validation analysis showing range of *R. padi* densities over number of sample taken for Green's sequential sampling plan.

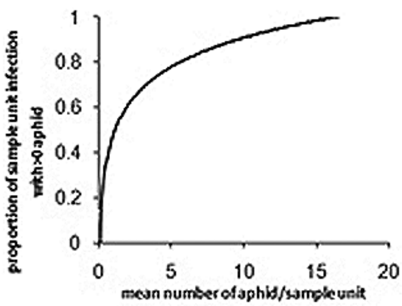

Fig. 6. Relation between the proportion of sampling units (4 stems) that had one or more (i.e., >0) individuals of aphids, and the mean number of aphids per sample unit.

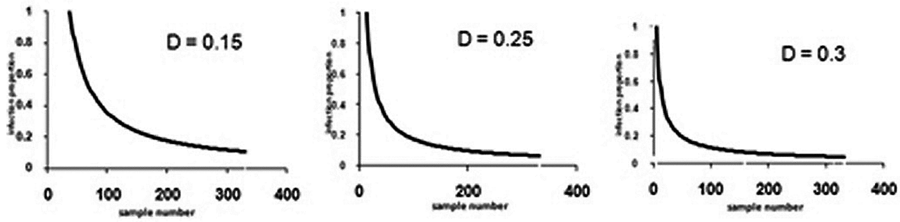

Fig. 7. Number of required samples for estimating the population density of *R.padi* in precision levels of 0.15, 0.25 and 0.3 in the fields of Badjgah based on Wilson and Room's model.

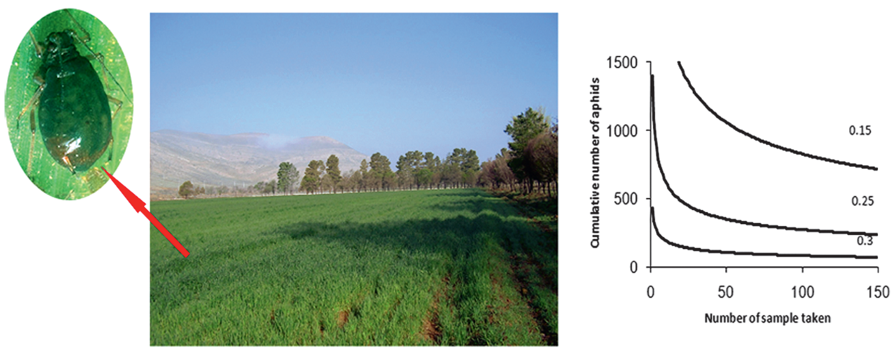

Fig. 8. Graphical Abstract.