J. Agric. Sci. Technol. (2009) Vol. 11: 27-38

Population Density and Spatial Distribution of *Aphis gossypii* Glover (Homoptera: Aphididae) on Cotton in Gorgan, Iran

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ABSTRACT

The seasonal abundance patterns of the cotton aphid, *Aphis gossypii* Glover, in cotton fields at Gorgan in northern Iran were studied during two growing seasons of 2002 and 2003. The spatial distribution of different developmental stages and morphs of the aphid was described by fitting data to Poisson (random) and negative binomial (aggregated) distributions, and calculating the dispersion indices. A sequential sampling plan was also developed using the fixed-precision method of Green for estimating the density of the adult, nymph and total population. The first aphid colonies appeared on plants during late June and early July and peaked in early September when cotton plants were at the boll maturation and opening stages. Aphid populations, especially nymphs and apterous females, were aggregated during most of the growing season and negative binomial models fit data sets better than the Poisson series. The percentage fit for alate morphs showed a slight tendency to the Poisson distribution. With respect to sampling cost or required sample size, the developed fixed-precision sequential sampling plans showed an acceptable performance for estimating aphid density at the precision level of D= 0.25. The optimum sample size was flexible and depended upon the aphid density and desired level of precision, and generally ranged from 10 to 513 and 62 to 3,206 at the precision levels of 0.25 and 0.10, respectively. The sequential sampling plans developed could be recommended to estimate the aphid density in integrated pest management programs.

Keywords: *Aphis gossypii*, Northern Iran, Seasonal fluctuation, Sequential sampling, Spatial distribution.

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[DOR: 20.1001.1.16807073.2009.11.1.10.9]

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is the most common aphid species occurring on the cotton *Gossypium hirsutum* L. in the northern cotton fields of Iran. The pest status of this aphid has changed in recent years and it has been considered as a serious pest in main cotton production areas of Iran (Darvish-Mojeni and Rezvani, 1997; Afshari *et al*., 2006).

The spatial pattern is an intrinsic feature of the species and results from the interaction between the insects and their habitat and, hence, may reflect behavioral characteristics of the species (Taylor, 1984; Kuno, 1991). There has been some effort to describe the spatial distribution of *A. gossypii* on cotton (Denechere, 1981; Zhang *et al*., 1998; Celini and Vaillant, 2004) and other crops such as strawberry, cucumber, melon and watermelon (Trumble *et al*., 1983; Burgio *et al*., 1994; Burgio and Ferrari, 1996) and aggregated dispersion has been reported for aphid populations on these crops. In comparative studies of models, Taylor's power model was usually found to fit the spatial dispersion better than Iwao's model (Celini and Vaillant, 2004; Kapatos *et. al*., 1996).

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Seasonal fluctuations of *A. gossypii* and the effect of some abiotic and biotic factors such as environmental conditions, agronomic practices and natural enemies on its population dynamics have been studied in the world's main cotton production areas (Kern and Gaylor, 1993; Slosser *et al*., 1998; Xia *et al*., 1999; Cisneros and Godfrey 2001). The use of density and spatial distribution information to develop a sampling program, especially sequential sampling, has been reported for the *A. gossypii* population on cotton (Zhang *et al*., 1998) and other crops such as melon and watermelon (Burgio *et al*., 1994; Burgio and Ferrari, 1996).

Despite the importance of cotton aphids in Iran, adequate information on the spatial distribution and reliable sampling plans has not been developed for this aphid in cotton fields.

The main objectives of this study were: (1) to determine the population density and seasonal dynamics of *A. gossypii* on cotton in northern Iran, (2) to describe the within-field distribution characteristics of the cotton aphid population and the effects of plant strata and aphid developmental stages on population dispersion characteristics, and (3) to develop fixed-precision sequential sampling plans (Green, 1970) for estimating *A. gossypii* density on cotton.

MATERIALS AND METHODS

General

Research was conducted in two experimental fields at Hashem-Abad Research Station, of the Iranian Cotton Research Institute in Gorgan, northern Iran (36º 55´ E and 54º 20´ N), during two growing seasons of 2002 and 2003. The fields were sown with the cotton (*Gossypium hirsutum* L.) cultivar Sahel, a normal leaf cultivar, on 27 May in 2002 and 22 May in 2003 and ranged in size from 1 to 2 ha (≈ 62500 plants per ha, 80 cm row widths). Cultural practices were carried out according to normal practice and no pesticides were applied during the study period. All samples were taken between 8 and 11 am. The average minimum/maximum temperatures during sampling dates were 23.5/33°C and 22.5/32°C, in 2002 and 2003 growing seasons, respectively.

Sampling Procedure

Samples were taken twice weekly on 23 dates from 30 June to 18 September 2002 and 25 dates from 29 June to 26 September 2003. The fields were divided into 10 blocks of the same size; then, inside each block, plants were randomly selected along a diagonal transect and three leaves from the upper, middle and bottom strata of the plants were taken randomly on side branches from each plant. The number of leaves (n) sampled on each sampling date was calculated using equation 1 (Southwood, 1995)

$$n = \left(\frac{ts}{d\bar{x}} \right)^2 \quad (1$$

Where $\bar{x}$ is the mean density of aphid per leaf, s is standard deviation, d is sampling error level, which was set to 0.25 in this study and t is Student's t of standard statistical tables (1.96 for more than 10 samples at 5% level). The maximum sample number was set to 150 leaves (truncated sampling). As a result, sample sizes ranged from 15 to 150 leaves or 5 to 50 plants during two sampling years. Leaves were transported to the laboratory and the number of aphid developmental stages was counted separately under the stereomicroscope.

Spatial Distribution

Both the negative binomial and Poisson distributions were fitted to the data obtained on each sampling date. The goodness of fit was determined using Chi-square test and T-statistic at the 0.05 level (Poole, 1974; Southwood, 1995). Several indices, commonly used to describe the distribution of *A. gossypii* in cotton fields, were also calcu-

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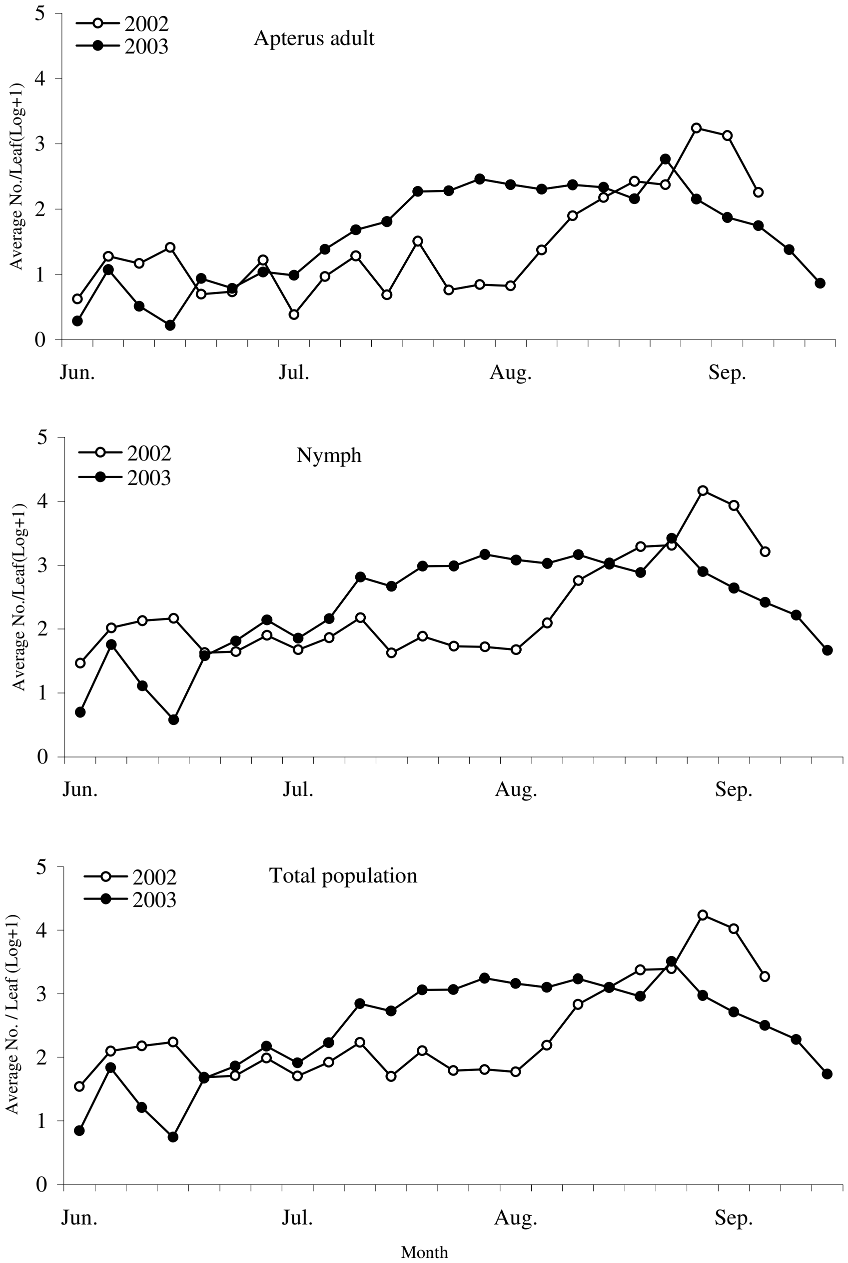

Figure 1. Seasonal fluctuation of apterus adult, nymph and total populations of *A. gossypii*, in northern cotton fields of Iran, during the 2002 and 2003 growing seasons.

lated. These include the ratio of variance/mean (Poole, 1974; Taylor, 1984; Southwood, 1995), *k* parameter of the negative binomial distribution (Bliss and Fisher, 1953; Southwood, 1995), Morisita's index (Poole, 1974; Young and Young, 1998) and the *b* exponent of Taylor's power law (Taylor, 1961). A t-test (Tsai et al., 2000) was used to determine if *b* was significantly different from 1.

The estimates of the dispersion parameter *k* were linearly regressed on population

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mean (m) as equation 2, to test for the existence of a common k (kc) for each year and overall data sets (Feng and Nowierski, 1992b; Tsai et al., 2000).

$$k = c + dm \quad (2$$

A d value significantly >0 indicates the dependence of k on mean density and justifies estimation of a common k. Estimates of kc were made using a regression method (Southwood, 1995; Tsai et al., 2000), which estimates kc by regressing $y' = s^2 - \bar{x}$ on $x' = \bar{x}^2 - (s^2/N)$, and kc was defined by $kc = 1/slope$ (Southwood, 1995; Tsai et al., 2000).

Sampling Plan Development

The optimum sample sizes necessary for estimating population density of *A. gossypii* at two levels of fixed precision (0.25 and 0.10) were calculated by the equation 3 (Buntin, 1994):

$$n = a\bar{x}^{(b-2)} / D^2 \quad (3$$

Where n is required sample size, $\bar{x}$ is mean density of aphid per leaf, D is a fixed level of precision, and a and b are coefficients obtained from the Taylor's power law regression.

The critical stop lines, the number of samples after which sampling can be terminated, for fixed-precision sequential sampling plans were calculated using equation 4 (Greens, 1970):

$$T_n = (an^{1-b} / D^2)^{1/(2-b)} \quad (4$$

Where T_n is the critical cumulative number of individuals counted in n samples, a and b are coefficients obtained from the Taylor's power law regression and D is the required level of precision, measured as $D = s_{\bar{x}} / \bar{x}$, where $s_{\bar{x}}$ is the standard error of $\bar{x}$. A precision level of 0.25 is generally accepted for pest sampling in IPM programs, while a precision level of 0.10 is generally desired for research purposes (Southwood, 1995); thus, we chose these two precision levels for use in this study.

Statistical Analysis

SAS software (SAS institute, 1997) and analysis of variance (ANOVA) were used to determine the effect of plant strata and aphid stages/morphs on dispersion indices. A *t*-test (Feng and Nowiersky, 1992 a, b) was used to compare the b parameter of Taylor's power law between years, aphid developmental stages and plant strata.

RESULTS

Population Density and Fluctuation

The first aphids colonized cotton plants in late June or early July, were low throughout the early season, began to increase in August, and peaked in early September (Figure 1). The aphid population peaked on 7-11 September during both 2002 and 2003, when cotton plants were at the boll maturation and opening stages (Figure 2). The aphid population exhibited some other distinct peaks with lower densities during August in 2003 (Figure 1), when cotton plants were at the squaring-flowering stage (Figure 2). Early-season aphid population densities which develop on cotton seedlings and pre-squaring stages were usually low during the two years of the study and reached a maximum number of 15.04±4.06 aphids per leaf (Figure 1).

The mean (±SE) peak densities of apterus adult, nymph and total population of *A. gossypii* were estimated to be 174.1±34.5, 1460.3±232.9 and 1724±269 individuals/leaf, respectively, during 2002 and 58.4±12, 263.4±64.2 and 324.3±75.4 individuals/leaf, respectively during 2003. Mean population densities of *A. gossypii* were not significantly different among the strata of the cotton plant (upper, middle and bottom) for nearly all of the sampling dates during the growing season (ANOVA, P> 0.05).

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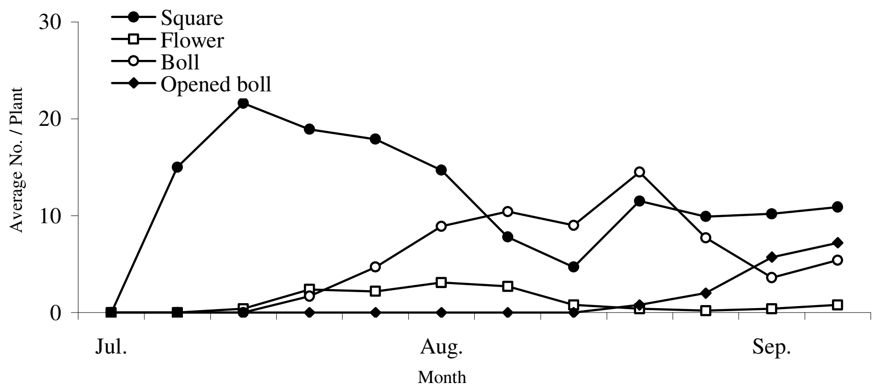

Figure 2. Seasonal appearance of cotton reproductive organs, in northern cotton fields of Iran at Gorgan.

Within-field Spatial Distribution

Distribution Models

Data sets from counts of different developmental stages and morphs of *A. gossypii* fit the negative binomial better than the Poisson series (Table 1). When T-statistics were used for spatial analysis, 87.5 percent of total population data sets fit the negative binomial distribution. The percentage fit of the negative binomial was obviously reduced (16.7 percent) when the Chi-square test was used to test the fit between observed and expected frequencies. The fitness percentage of alate morphs revealed a slight tendency towards the Poisson distribution, so that 50 and 20.8 percent of data sets from alate populations fit the Poisson distribution in Chi-square and T-statistic analysis, respectively (Table 1).

Dispersion Indices

Taylor's power law: Taylor's regression of log S^2 on $\log \bar{x}$ provided a good fit to the data from different *A. gossypii* stages and morphs and the values of r^2 ranged from 0.867 to 0.955 (Table 2). Taylor's *b* value for the two growing season data sets was significantly greater than 1 in three strata of the cotton plant, indicating that the distribution of all stages and morphs on cotton plant were contagious (Table 3).

Although *b* values for all stages and morphs were greater than 1, *t-tests* results revealed that the smallest *b* on three plant strata was for alate morphs (df= 86, t= 2.22,

Table 1 . Percentage fit of counts of *A. gossypii* different stages or morphs to negative binomial and Poisson frequency distributions, using two *Chi-square* and *T-statistics* goodness of fit tests.

	Goodness of fit test			
	Chi-square		*T-Statistics*	
Stage/Morph	NB[a]	P[b]	NB	P
Adult-apterae	47.9	04.2	93.8	06.2
Adult-alatae	83.3	50.0	79.2	20.8
Nymphs	18.7	00.0	81.2	17.8
Total	16.7	00.0	87.5	12.5

[a] Negative binomial, [b] Poisson.

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Table 2. Regression statistics of Taylor's power law for different stages and morphs of *A. gossypii* populations at upper, middle and lower strata and whole plant.

Plant strata	Aphid stage/morph			
	Adult (apterae)	Adult (alatae)	Nymph	Total
Upper				
n	48	17	47	47
r^2	0.885	0.902	0.887	0.877
p	< 0.001	< 0.001	< 0.001	< 0.001
Middle				
n	47	19	47	47
r^2	0.938	0.867	0.913	0.955
p	< 0.001	< 0.001	< 0.001	< 0.001
Lower				
n	47	16	47	47
r^2	0.937	0.928	0.913	0.923
p	< 0.001	< 0.001	< 0.001	< 0.001
Whole plant				
n	47	24	47	47
r^2	0.900	0.931	0.904	0.870
p	< 0.001	< 0.001	< 0.001	< 0.001

Table 3. Taylor's regression coefficients for different stages and morphs of *A. gossypii* populations, at upper, middle and lower strata and whole plant.

Plant strata	Aphid stage/morph			
	Adult (apterae)	Adult (alatae)	Nymph	Total
Upper				
Log a±SE	0.88±0.07	0.46±0.09	1.09±0.13	1.04±0.19
b±SE	1.54±0.08	1.42±0.12	1.61±0.07	1.66±0.12
Middle				
Log a±SE	0.89±0.06	0.42±0.10	1.13±0.12	1.10±0.21
b±SE	1.60±0.06	1.42±0.13	1.62±0.08	1.61±0.13
Lower				
Log a±SE	0.84±0.05	0.41±0.09	1.15±0.10	0.98±0.14
b±SE	1.48±0.58	1.31±0.10	1.48±0.07	1.57±0.09
Whole plant				
Log a±SE	1.08±0.07	0.49±0.08	1.40±0.11	1.38±0.13
b±SE	1.49±0.07	1.27±0.07	1.51±0.07	1.51±0.21

P< 0.05 and df= 86, t= -2.42, P< 0.05), indicating that alate adults were significantly less clumped in their distribution than apterous adults and nymphs. This supports the findings of discrete frequency distribution analysis.

Ratio of variance/mean $(s^2/\bar{x})$: The variance/mean ratio of nymphs and total population were always significantly greater than 1 while, in some data sets of apterous and alate adult populations, the ratio was significantly less than 1. Hence, 2.1 and 41.7 percent of data sets showed a ratio significantly less than 1 in apterus and alate adult populations, respectively (Table 4).

k-parameter: The negative binomial parameter (*k*) for the aphid population ranged from 0.019 to 0.557 (Figure 3). This indicates that the populations of *A. gossypii* were generally aggregated on cotton plants during both growing seasons (Table 4). There were no significant differences in *k* values of the plant stratum (F= 1.99, df= 2, P= 0.138) but

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Table 4 . Percentage fit of counts of *A. gossypii* different stages or morphs from cotton fields to aggregated and random dispersions, according to three dispersion indices.

Stage/morphs	Dispersion Indices					
	Variance/Mean		Morisita's Index		*k*	
	Aggre.[a]	Ran.[b]	Aggre.	Ran.	Aggre	Ran.
Adult-apterae	97.9	02.1	100	00.0	97.9	02.1
Adult-alate	58.3	41.7	50.0	50.0	72.9	27.1
Nymphs	100	00.0	100	00.0	100	00.0
Total	100	00.0	100	00.0	100	00.0

[a] Aggregated dispersion, [b] Random dispersion.

there were significant differences of aphid developmental stages/morphs (F= 5.77, df= 4, P= 0.0002). The values of *k* for apterous and alate adult were generally higher than those of the nymph and total populations. Thus, based on *k* values, 2.1 and 21.8 percent of apterous and alate adult sampling data sets deviated from aggregated dispersion (k>8)during two years of the study (Table 4).

Morisita's index (I_δ): Seasonal variability of I_δ obtained for the total population data sets ranged from 1.34 to 46.1 (Figure 3). Contrary to *k*, the highest values of I_δ were more frequently observed during the early season when aphid density was generally low. The values of I_δ decreased gradually during the growing season and reached their lowest value in late season. Although, in terms of total population, *A. gossypii* were aggregated during growing season, alate forms showed a random dispersion at 50 percent of data sets according to this index (Table 4). There were no significant differences among I_δ values of plant stratum, but there were significant differences among aphid

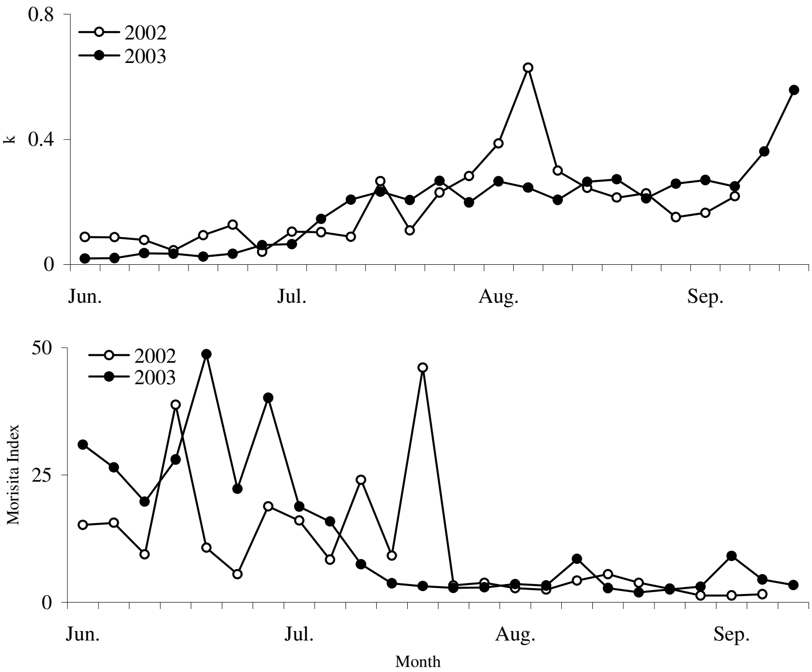

Figure 3. Seasonal variability of *k* and Morisita's aggregation index (I_δ) in cotton fields of Gorgan, Iran, during the 2002 and 2003 growing seasons.

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stages/morphs (F= 8.37, df= 4, P= 0.0001).

Estimation of Common *k* (k_c)

The slopes of the regression of *k* on the mean density per leaf (*m*), were not significantly greater than 0 for the 2002, 2003 and pooled data sets (Table 5). Independence of *k* from the mean density indicates the existence of a common *k* for the negative binomial distribution of the aphid population (Table 5). The value of k_c ranged from 1.625 of alate adults to 3.058 of total population.

Sequential Sampling Plans

Optimum Sample Size (OSS) and Critical Stop Lines: Optimum ample size curves for fixed levels of precision are shown in Figure 4 A-C. At a given level of precision, the optimum sample size decreased rapidly as aphid density increased for both adult and nymph populations. For example, at a precision level of 0.25 that is proper for IPM purposes, the OSS varied from the minimum of 10 leaves, which estimated in late season when total population density was 1724 aphids/leaf, to a maximum of 513 in early season when population density was 0.553 aphids/leaf.

To achieve a desired precision level of 0.10 for research purposes, higher sample sizes were required than at a precision level of 0.25, so that the minimum and maximum of required sample size increased to 62 and 3,206, respectively (not shown in the figure). Although such sample sizes could be acceptable for research purposes, they are not practicable for agronomic or pest management purposes, because of the high sampling cost.

From the sample size results, it can be concluded that the developed fixed-precision sequential sampling plans will be useful and time-effective for estimating aphid density at the level of precision D= 0.25. At the higher level of precision (e.g. 0.10), they required quite large sample sizes, especially at the low densities of early season.

One attribute of OSS is that, at a given density, the required sample size increases as the dispersion pattern becomes more aggregated (Wipfli *et al*., 1992). Thus, with a given level of precision, fewer numbers of samples are always required for estimation of adult density than for nymph and total population (Figure 4A-C). For example, at the mean density of 15 individuals per leaf, a total of 48, 106 and 102 samples (leaves)

Table 5. *P* and *F* values of regression between aggregation index, *k*, (dependent variable) and aphid mean density (independent variable) and estimated common *k*, k_c, during the 2002 and 2003 growing seasons.

Studying years	Aphid stage/morph			
	Adult (apterae)	Adult (alatae)	Nymph	Total
2002				
F	0.142	0.478	0.039	0.031
p	0.710	0.497	0.846	0.864
k_c	1.706	1.661	2.570	3.033
2003				
F	0.247	3.720	3.085	2.28
p	0.624	0.066	0.092	0.144
k_c	0.685	0.309	0.466	0.515
Overall				
F	0.166	0.791	0.202	0.009
p	0.685	0.378	0.655	0.924
k_c	1.701	1.626	2.570	3.058

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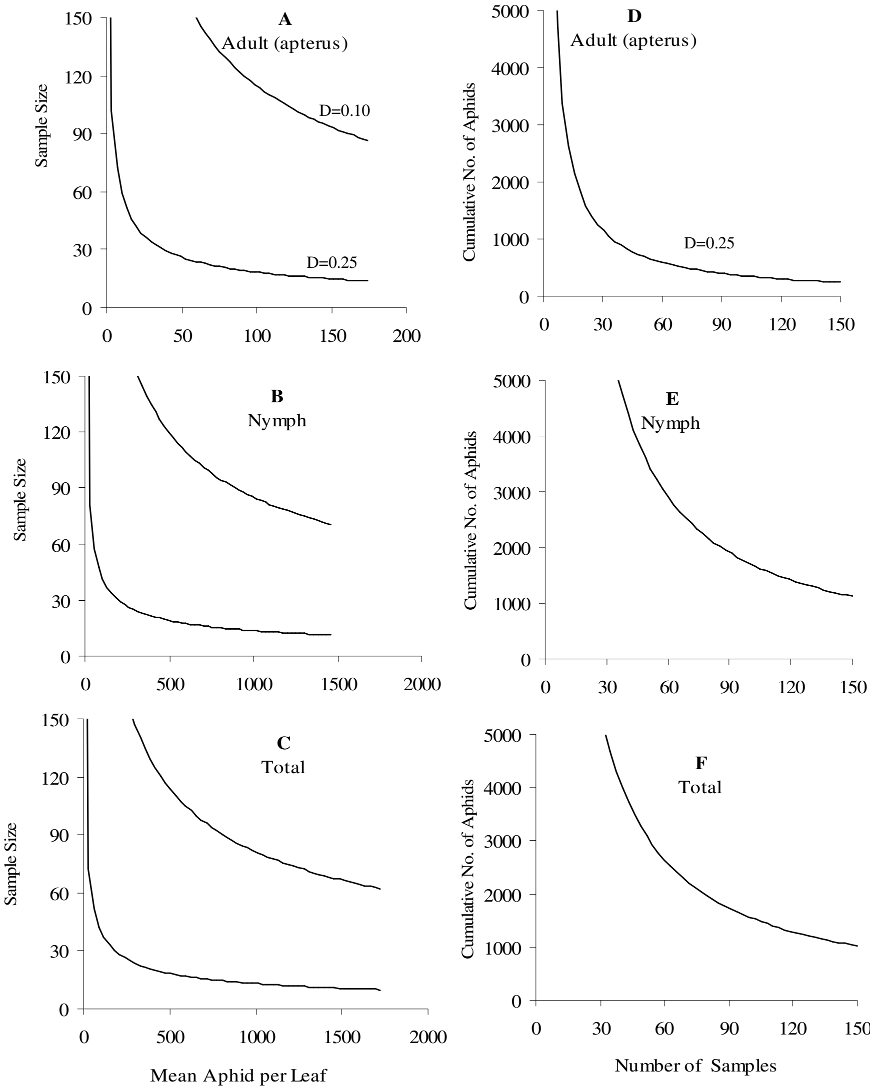

Figure 4. Sample size curves (A-C) and stop lines for fixed-precision sequential sampling (D-F) for nymph, adult and total population of *A. gossypii* at precision levels of 0.10 and 0.25.

would be required to provide reliable estimates of adult, nymph and total population, respectively, at the level of precision D= 0.25.

The critical stop lines of the sequential sampling plans for the cumulative number of adult, nymph and total population are shown in Figure 4D-F. The stop line of the fixed-precision level of 0.10 was not shown because of the need for extremely large samples from the field. The number of sample units required to cross the stop lines decreased with an increase in the aphid density.

The developed sequential plans are executed practically by randomly selecting samples (leaves) and counting the number of aphids. The cumulative total number of aphids (T_n) collected in n leaves is plotted on a graph as samples are accumulated. If the point (n, T_n) is below the line for the desired level of precision (D), it is necessary to continue sampling because the fixed level of precision has not yet been reached. On the

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contrary, when it is above the line for the desired level of precision (*D*), the mean can be estimated with T_n/n and the desired level of precision has been obtained.

DISCUSSION

The mean density of *A. gossypii* in our study was clearly higher than in the previous study (Darvish-Mojeni and Rezvani, 1997). Developing resistance to conventional insecticides (Bayat-Assadi and Porghaz, 1999), climatic changes and a reduction in natural enemies' population may play an important role in this increase.

Peak seasonal *A. gossypii* infestations in the cotton fields of northern Iran during the two years of sampling occurred in late season. Most often, late season outbreaks of the aphid population has destructive effects on lint quality parameters, whereas mid-season minor outbreaks, as occurred in 2003, significantly reduce cotton reproductive organs and lint yields (Afshari *et al*., 2006).

There are some studies that describe the spatial distribution of *A. gossypii* on cotton (Zhang *et al*., 1998; Celini and Vaillant, 2004) and other crops such as cucumber, melon and watermelon (Burgio *et al*., 1994; Burgio and Ferrari, 1996), using dispersion indices that only show the aggregation or randomness of behavior of the aphid population. In this study, we examined in addition the fitness of the population data of the *A. gossypii* to the negative binomial and Poisson frequency distribution in order to obtain the mathematical distribution models.

The suitability of Taylor's regression model for describing the dispersion of *A. gossypii* has been reported on cucumber (Burgio *et al*., 1994), watermelon (Burgio and Ferrari, 1996) and cotton (Celini and Vaillant, 2004). Our estimate of *b* was not significantly different from those calculated by Burgio *et al*. (1994) on cucumber (t= 0.936, P> 0.2), and Celini and Vaillant (2004) on cotton (t= 0.898, P> 0.2).These results confirm the within-species constancy of *b* and justify its application in the estimation of the spatial distribution of cotton aphids.

Stratum-based sampling of *A. gossypii* has been recommended in some studies (Kern and Gaylor, 1993; Hollingsworth *et al*., 1995; Celini and Vaillant, 2004), when there were significant differences in aphid density within a plant stratum. Our results showed that there were no significant differences in aphid dispersion and mean density within any plant stratum. Therefore, in order to reduce sampling costs, cotton leaves can be chosen randomly, regardless of their position or stratum on plant.

In this study we developed a fixed-precision sequential sampling plan for estimating the density of cotton aphids in northern Iran. Similar fixed sequential sampling plans have been developed for this aphid on several crops, including cotton (Zhang *et al*., 1998) and cucumber (Burgio *et al*., 1994). Our results showed that Green's fixed-precision sequential sampling plans are acceptable and cost-effective for estimating aphid density at the precision level of 0.25. To achieve a higher precision level for research applications, extremely large samples must be taken that make the sampling plans impractical with respect to sampling cost and efficiency.

This sampling program should be useful for population ecologists studying cotton aphids and may be of use in a monitoring program for determining the need for control measures against cotton aphids.

ACKNOWLEDGEMENTS

This research was funded, in part, by the Iranian Cotton Research Institute. We gratefully acknowledge the support of Professor Hoshang Bayat-Assadi, the late Head of this Institute, to this project. We would also like to thank Dr. Roger Laughlin, from Australia, for his thoughtful review of the manuscript.

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تراکم و پراکنش فضایی جمعیت شته سبز پنبه (*Aphis gossypii* Glover, Homoptera: Aphididae) روی پنبه در گرگان، ایران.

ع. افشاری، ا. سلیمان‌نژادیان و پ. شیشه‌بر

چکیده

در طول سالهای ۱۳۸۱ و ۱۳۸۲ تغییرات فصلی جمعیت شته سبز پنبه (*Aphis gossypii* Glover) در مزارع پنبه گرگان، شمال ایران، مورد بررسی قرار گرفت. پراکنش فضایی مراحل و شکلهای مختلف رشد شته با استفاده از دو مدل پویسون و دوجمله‌ای منفی و نیز براورد شاخصهای پراکنش، مورد ارزیابی قرار گرفت. بعلاوه، به منظور براورد تراکم جمعیت شته با یک دقت معین، اقدام به طراحی نمونه‌برداری دنباله‌ای با دقت ثابت (مدل گرین) برای جمعیت افراد بالغ، پوره‌ها و جمعیت کل شد. اولین کلنی شته در اواخر خرداد روی بوته‌های پنبه ظاهر شده و جمعیت شته در اواسط شهریور هنگامی که بوته‌های پنبه در مرحله رشد کامل و باز شدن قوزه‌ها بودند، به اوج خود رسید. جمعیت شته بویژه ماده‌های بدون بال و پوره‌ها در طول بخش زیادی از فصل زراعی دارای پراکنش تجمعی بوده و میزان برازش پراکنش آنها با مدل دوجمله‌ای منفی (تجمعی) بمراتب بیشتر از مدل پویسون (تصادفی) بود. جمعیت شته‌های بالغ بال‌دار از تمایل محسوسی برای نشان دادن توزیع تصادفی برخوردار بودند. طرحهای نمونه‌برداری دنباله‌ای طراحی شده در این بررسی از نظر هزینه نمونه‌برداری (تعداد نمونه لازم برای براورد تراکم)، از عملکرد قابل قبولی در براورد تراکم شته با دقت ۰/۲۵ برخوردار بودند. تعداد نمونه لازم برای براورد تراکم جمعیت شته متغیر بوده و به تراکم شته و دقت مورد نظر در براورد میانگین بستگی داشت. در دقتهای ۰/۲۵ و ۰/۱۰، تعداد نمونه‌ی بهینه بترتیب در محدوده ۱۰تا ۵۱۳ و ۶۲تا ۳۲۰۶ برگ متغیر بود. طرحهای نمونه‌برداری دنباله‌ای ارائه شده در این مطالعه برای استفاده در مدیریت تلفیقی آفات قابل توصیه می‌باشند.

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