# Sampling Procedure and Temporal-Spatial Distribution of the Cabbage Aphid, Brevicoryne brassicae L. (Hemiptera: Aphididae), on Canola 

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#### Abstract

To estimate population density of the cabbage aphid, Brevicoryne brassicae (L.), a stratified random sampling was conducted in two unsprayed canola fields in Isfahan (central Iran) during 2011-2012. Population density was determined for apterous, and alates, as well as the total population, at two plant growth phases (PGP1: From plant emergence to the end of rosette, and PGP2: From the beginning of stem elongation to ripening) on the whole plant as the sampling unit. Sources of variation in the sampling procedure were analyzed with a nested analysis of variance (NANOVA). In PGP2, aphid density in upper ( $10-15 \mathrm{~cm}$ upper part of stem) and lower (the rest of stem) parts were compared using Student's $\boldsymbol{t}$-test. Temporal changes in spatial pattern during the growth season were evaluated using $1 / k$ (aggregation index) and Lloyd's Patchiness Index. Results showed that differences among fields accounted for the majority of total variation observed in aphid densities and the aphids significantly preferred upper parts of canola plants. Among different indices used for analyzing spatial distribution of the aphid, Taylor's Power Law (TPL) described well the relationship between variance and mean of the population. In both PGPs spatial patterns of apterous, alates, and total population were aggregated, random, and aggregated, respectively. Estimates of $1 / k$ and changes in the value of patchiness index revealed that the aphid population was aggregated at the beginning of rosette stage and became more dispersed with time.


Keywords: Brassica napus, Between-plant distribution, Isfahan, Population density, Withinplant distribution.

## INTRODUCTION

The cabbage aphid, Brevicoryne brassicae (L.) (Hemiptera: Aphididae) is a specialist on Brassicaceae and prefers feeding on tender plant tissues. This makes the aphid a pest of economic importance on brassica crops as it can move into the developing flowers and render the crops unmarketable (Costello and Altieri, 1995). In Iran, damage due to the cabbage aphid is usually so
serious that canola, Brassica napus L., crops require insecticide treatments to ensure profitable production and reduce economic damage (Behdad, 1996).
Stratified sampling can improve sampling efficiency by reducing sample variation for a given sampling effort and by ensuring that samples are collected from all areas of the habitat (Buntin, 1994). Knowledge of an insect's distribution pattern provides an informative description of a population

[^0](Iwao, 1968). It can also affect the sampling program and the method of analysis of the data (Southwood and Henderson, 2000).

Cabbage aphid is among the most serious pest insects of canola production in Iran and the development of a sound sampling procedure for accurate density estimation is a prerequisite for integrated pest management programs. The present study aimed to develop a sampling procedure suitable for population dynamics studies of the cabbage aphid on canola, and to determine the within- and between-plant distributions and temporal changes in spatial pattern of the aphid in canola fields.

## MATERIALS AND METHODS

## The Study Site

The experiment was conducted in Baraan region ( $32^{\circ} 30^{\prime} 34^{\prime \prime} \mathrm{N}$ and $51^{\circ} 49^{\prime} 57^{\prime \prime} \mathrm{E}$, at $1,547 \mathrm{~m}$ altitude) of Isfahan county (central Iran), during 2011-2012. The experimental fields were in a typical area of commercial canola production. Canola, cv. Okapi (the most common cultivar grown in Isfahan province), was sown in two separate fields on $29^{\text {th }}$ September 2011, i.e. early enough to become naturally infested with immigrant alate aphids. Each field was $500 \mathrm{~m}^{2}$, and divided into two equal blocks. Recommended cultural and agronomic practices were adopted from sowing to harvest. No insecticide was sprayed in and around the experimental fields. To encourage uniform aphid colonization, each field was surrounded by nearly 30 m wide fallow area.

## Sampling Procedure

A stratified random sampling scheme was used to estimate aphid numbers. The sampling unit was a whole plant and, based on a preliminary sampling, ten samples were randomly taken within each block (20 plants/field). Samples were taken weekly
and a total of 1,280 plants were sampled in a 32 -week period (October 2011-June 2012). Growth stages of the crop were recorded using the key provided by Harper and Berkenkamp (1975), with minor modifications. Aphid population density was recorded in two plant growth phases, PGP: from plant emergence to the end of rosette stage (PGP1), and from the beginning of stem elongation to ripening (PGP2). Population density of the aphid was then determined for three different aphid groups: apterous ( $1^{\text {st }}-4^{\text {th }}$ instars and apterous adults), alates (alate $4^{\text {th }}$ instars and alate adults), and total population.

Aphids were heat-extracted using the method described by Raworth et al. (1984). Before heat extracting, any active insects, including adult alate aphids and adult parasitoid and hyperparasitoid wasps, were collected. The aphid species in the sample were separated, and then all individuals of the cabbage aphid were separated by instars (Raworth et al., 1984) and counted. In samples containing more than 3,000 aphids, numbers were estimated using a volumetric sub-sampling technique and ratio estimation as described by Raworth et al. (1984). This technique was accurate when sub-sampling was unbiased with respect to stage/morph distribution, and the ratio of the aphids between the sub-samples and the main sample was adjusted. To check the former, stage/morph distribution in three subsamples were compared with that of the main sample, by means of Chi-square and Bonferroni Chi-square; and to check the latter, a linear regression was used between sub-samples and main sample.

The data were normalized using the log $(x+0.5)$ transformation. Sources of variation in aphid density must be identified to design an efficient sampling program. Therefore, a nested analysis of variance (NANOVA) was calculated for each data set to determine the relative importance of each source of variability, including dates and fields (data sets), blocks per field, and plants per block, in the hierarchical sampling design used in this study.

## Population Density and Within-plant Distribution

Mean number of aphid settling at different plant growth stages were recorded for the three aphid groups. In PGP2, aphid density on each plant was recorded in upper (10-15 cm upper part of the stem) and lower parts (the rest of the stem), and aphid preference for these parts was analyzed using Student's $t$-test. Mean demand ( $\mathrm{c}^{*}$ ), representing the mean number of individuals per sampling unit per individual (Lloyd, 1967), was calculated for different stages of plant growth.

## Between-plant Distribution

To analyze the spatial distribution, means (m) and variances $\left(\mathrm{s}^{2}\right)$ for count of aphid per plant were calculated for each sampling date. Spatial distribution of the aphid was determined using 7 distribution indices, including the variance to mean ratio ( $\mathrm{s}^{2} / \mathrm{m}$ ) (Southwood and Henderson, 2000), Green's index (Green, 1966) and standardized Morisita's index (Ip) (Smith-Gill, 1975), which Myers (1978) considered as good measures of spatial distribution. Moreover, Lloyd's mean crowding index ( $\mathrm{m}^{*}$ ) (Lloyd, 1967) and Morisita's index of dispersion ( $\mathrm{I}_{\delta}$ ) (Morisita 1962) were calculated for each data set. Departures from randomness for these indices were assessed by the appropriate goodness of fit formulae (Davis, 1994). Further information on the pattern of distribution was obtained by two regression techniques: Taylor's Power Law (TPL) (Taylor, 1984) and Iwao's Patchiness Regression (IPR) (Iwao, 1968). Homogeneity of intercepts and slopes of TPL for apterous and alates between the two PGPs were evaluated using Student's $t$-test formulae (Feng and Nowierski, 1992): $t_{\text {intercept }}=\left(a_{l^{-}}\right.$ $\left.a_{2}\right) /\left(S E_{a l}^{2}+S E_{a 2}^{2}\right)^{1 / 2} \quad$ and $\quad t_{\text {slope }}=\left(b_{1^{-}}\right.$ $\left.b_{2}\right) /\left(S E_{b 1}^{2}+S E_{b 2}^{2}\right)^{1 / 2}$, where $d f=n_{1}+n_{2}-2,1$ and 2 are PGP1 and PGP2, respectively.

To evaluate temporal changes in spatial pattern of the aphid population during the
growing season, an aggregation index (1/k) (Southwood and Henderson, 2000) was used. The method described by Bliss and Owen (1958) was used to estimate the common K (Kc) for the three aphid groups in both PGPs. Because of the stability of TPL, the formula has been widely used to develop sampling program. Therefore, the optimum sample size was re-calculated using TPL coefficients obtained for the total population. Each table or figure has been provided with the corresponding used formulae in detail.

## RESULTS

## Sampling Procedure

Relative variation of the preliminary sampling was $2.28 \%$ and $2.49 \%$ for PGP1 and PGP2, respectively, which were less than the $10 \%$ recommended by Southwood and Henderson (2000). The calculated sample sizes for PGP1 and PGP2 were 19.99 and 19.18 , respectively. The stage/morph distributions in the sub-samples were statistically different, but the differences were not consistent with respect to instars (Table 1). The following regression equation was used to adjust the ratio between volumes of sub-samples and main sample: $Y=-0.343+1.12 X,\left(\mathrm{r}^{2}=0.991, \mathrm{df}=5\right)$, where $Y$ is sub-sample volumes and $X$ is main sample volume. The NANOVA analyses for both PGPs (Table 2) showed that differences among fields (data sets) accounted for the majority of total variation in aphid densities; $96 \%$ in PGP1 and $88 \%$ in PGP2. Blocks/field and plants/block/field contributed little to total variation. Therefore, these strata could be incorporated into above stratum, i. e. fields.

## Population Density and Within-plant Distribution

Population density of the aphid changed remarkably during the growing season. Significant differences were found for

Table 1. Comparison of proportions of stage/morph distribution of the cabbage aphid, Brevicoryne brassicae in sub-samples with proportions of stage/morph distribution in the main sample.

| Developmental stages/morphs | Replication one |  |  |  | Replication two |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Subsamples |  |  | Main sample | Subsamples |  |  | Main sample |
|  | 1 | 2* | 3 |  | 1* | 2* | 3* |  |
| $1^{\text {st }}$ instar | 0.503 | 0.561 a | 0.512 | 0.516 | 0.547 | 0.505 | 0544a | 0.499 |
| $2^{\text {nd }}$ instar | 0.227 | 0.249 b | 0.237 | 0.217 | 0.163 a | 0.202 | 0.224 | 0.256 |
| $3{ }^{\text {rd }}$ instar | 0.113 | 0.028 | 0.100 | 0.102 | 0.065 | 0.075 a | 0.080 | 0.96 |
| $4^{\text {th }}$ instar | 0.037 | 0.066 c | 0.053 | 0.057 | 0.064 | 0.053 | 0.038 | 0.050 |
| Alate $4^{\text {th }}$ instar | 0.015 | 0.003 | 0.006 | 0.012 | 0.021 ab | 0.015 | 0.012 | 0.009 |
| Apterous adult | 0.066 | 0.035 | 0.056 | 0.048 | 0.100 b | 0.079 a | 0.022 | 0.047 |
| Alate adult | 0.056 | 0.056 bc | 0.034 | 0.045 | 0.054 | 0.068 | 0.076 b | 0.043 |
| Total aphid | 316 | 285 | 320 | 3322 | 367 | 465 | 312 | 3523 |

[^1]Table 2. Results of NANOVA computed for total population density of the cabbage aphid, Brevicoryne brassicae on canola plant, Isfahan, Iran, 2011-2012.

| PGP $^{a}$ | Variance source | df | Variance component | \% of total variation |
| :---: | :---: | :---: | :---: | :---: |
| PGP1 | Field |  |  |  |
|  | Block/Field | 41 | 59.322 | 96.292 |
|  | Plant/Block/ Field | 42 | 0.436 | 1.173 |
|  | Total | 756 | 0.077 | 2.534 |
| PGP2 | Field | 239 | 21 | 1.590 |
|  | Block/Field | 22 | 0.101 | 88.353 |
|  | Plant/Block/Field | 396 | 0.105 | 5.732 |
|  | Total | 439 | 1.769 | 5.912 |
|  |  |  | 100 |  |

[^2]population density of the three aphid groups among canola growth stages (Figure 1). The highest density was found in the stem elongation stage, whereas the lowest density occurred in the ripening and rosette stages without significant difference. Population density of alates was always lower than apterous and accounted for only $3.67 \%$ of the total population throughout the plant growth period. The rosette stage, or PGP1 accounted for $22.7 \%$ of the total population throughout the plant growth. The highest aphid density was found in mid stem elongation and, afterward, it decreased gradually (Figure 2). In PGP2, 65.85 and $34.13 \%$ of the total population were found on the upper and lower plant parts, respectively. Student's $t$-tests of
the data sets indicated significant preference for the upper part of plants on all sampling dates and aphid groups (Table 3), except for alates in the last sampling date $(\mathrm{t}$-value $=1.49$, $\mathrm{df}=39, \mathrm{P} \geq 0.139$ ). Based on calculations of mean demand, the density of conspecific individuals per canola plant that a typical individual of the cabbage aphid could expect ranged from 89 to 26,808 individuals. It was highest in stem elongation and lowest in rosette stages (Table 4).

## Between-plant Distribution

Five non-regression spatial distribution indices (Table 5) showed that aphid


Figure 1. Mean number of apterous, alate and total population of the cabbage aphid, Brevicoryne brassicae in different canola growth stages. For each aphid group, bars with different letters are significantly different from each other, Duncan's multiple range tests at $P<0.05$.


Figure 2. Mean number of the cabbage aphid, Brevicoryne brassicae on upper and lower parts of canola plant in different plant growth stages. Plant growth stages and codes were based on the key provided by Harpper and Berkenkamp (1975).

Table3. Within-plant distribution of the cabbage aphid, Brevicoryne brassicae on canola in PGP2 ${ }^{a}$, Isfahan, Iran, 2011-2012.

| Aphid group | Aphids/Plant (Mean $\pm$ SE) |  | df | $\mathrm{pr}^{2} \mathrm{t}^{b}$ |
| :---: | :---: | :---: | :---: | :---: |
|  | Lower part of plant | Upper part of plant |  |  |
| Apterous | $3847.1 \pm 201.65$ | $7448.6 \pm 313.82$ | 439 | $<0.0001^{* *}$ |
| Alates | $127.73 \pm 3.62$ | $266.19 \pm 10.29$ | 439 | $<0.0001^{* *}$ |
| Total | $3974.8 \pm 204.76$ | $7714.8 \pm 322.94$ | 439 | $<0.0001^{* *}$ |

${ }^{a} 2^{\text {nd }}$ Plant Growth Phase: From the beginning of stem elongation to ripening.
${ }^{b}$ Significant differences between means (unequal variances), using paired Student's $t$-test at $P \leq 0.01$.
Table 4. Estimates of mean demand ${ }^{a}$ (mean $\pm$ SE) for the cabbage aphid, Brevicoryne brassicae in different plant growth stages of canola, Isfahan, Iran, 2011-2012.

| Plant growth stage | Mean $\pm$ SE for aphid groups |  |  |
| :---: | :---: | :---: | :---: |
|  | Apterous | Alates | Total population |
| Rosette | $1835 \pm 443.85$ | $89.68 \pm 8.65$ | $175.91 \pm 439.64$ |
| Stem elongation | $26808.09 \pm 2833.55$ | $812.80 \pm 81.16$ | $26018 \pm 2755.31$ |
| Flower | $10525.23 \pm 3024.12$ | $338.38 \pm 70.65$ | $10293.6 \pm 92959.24$ |
| Ripening | $1488.39 \pm 74.30$ | $165.19 \pm 26.12$ | $1331.68 \pm 48.88$ |

${ }^{a}$ Calculated by the formula of $c^{*}=m^{*}+1$, where $c^{*}$ is mean demand, and $m^{*}$ is Lloyd's mean crowding index (Lloyd, 1967).

Table 5. Range of five spatial distribution indices and percentage of aggregated data set ${ }^{a}$ for the cabbage aphid, Brevicoryne brassicae on canola, Isfahan, Iran, 2011-12.

| Index | Apterous |  |  | Alates |  | Total population |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\%$ | Range | $\%$ | Range | $\%$ | Range |  |
| $\mathrm{S}^{2} / \mathrm{m}^{b}$ | 100 | 2.07 to 163.39 | 100 | 1.68 to 30.89 | 96.87 | 0.89 to 160.50 |  |
| Lloyd's mean crowding $\left(\mathrm{m}^{*}\right)^{c}$ | 100 | 3.98 to 104.17 | 100 | 2.72 to 40.37 | 96.87 | -0.37 to 103.17 |  |
| Green's $\left(\mathrm{C}_{\mathrm{X}}\right)^{d}$ | 100 | 0.0 to 1.0 | 100 | 0.0 to 1.0 | 100 | -0.0 to 1.0 |  |
| Morisita's $\left(\mathrm{I}_{\boldsymbol{\delta}}\right)^{e}$ | 100 | 1.0 to 3.04 | 100 | 1.0 to 1.30 | 100 | 0.99 to 1.52 |  |
| Standardized Morisita's $(\mathrm{Ip})^{f}$ | 100 | -0.50 to -0.47 | 100 | -0.50 to -0.49 | 100 | -0.50 to -0.49 |  |

${ }^{\text {a }}$ Data set is sampling datexfield, ( $\mathrm{n}=40$ for each sampling date); ${ }^{\text {b-e }}$ For each index, departure from randomness for each data set was evaluated with a $z$-value (Southwood and Henderson, 2000 ); with a $z$-value (Lloyd, 1967); with an $x^{2}$ analysis (Green, 1966); with a $z$-value (Davis, 1994), respectively, ${ }^{f}$ The values range from -1.0 to 1.0 and the $99 \%$ confidence limits for randomness are standardized to -0.5 and 0.5 (Smith-Gill, 1975).
population had an aggregated distribution for all data sets, except total population for $s^{2} / m$ and $m^{*}$, for which nearly $4 \%$ of data sets had a random distribution. TPL provided a good fit of the data for different aphid groups, and $r^{2}$ values ranged from 0.51 to 0.95 (Table 6). Coefficient of ' $b$ ' indicated that the distributions of apterous and total population were aggregated. Alate aphid, however, showed a random pattern. Statistical tests for homogeneity revealed that the slopes and intercepts of TPL for apterous and alates did not differ between two PGPs $(\mathrm{df}=30, \mathrm{P}<0.05$; for apterous: $t$ value $_{a}=0.466, t-$ value $_{b}=0.014$; for alates: $t$ value $_{a}=0.619, t$-value $_{b}=-0.617$ ). Regarding $\alpha$ and $\beta$ parameters of the IPR, Davis (1994) defined three major types of aggregation patterns. Based on this grouping, all aphid groups, except apterous in PGP2, had dispersions with constant tendency for
aggregation $(\alpha=0, \beta>1)$. Apterous aphid in PGP2, however, had random distribution of clumps with constant clump size $(\alpha>0, \beta=$ 1) (Table 7).

The estimates of the aggregation index $(1 / k)$, with respect to mean aphid density at different plant growth stages were consistently more than zero for all 32 observations (Figure 3). It ranged from 0.0005 to 2.0869 for apterous, 0.005 to 0.310 for alates, and 0.0007 to 0.536 for total population. This index showed close aggregation at the rosette stage, especially for alates. However, from the beginning of the stem elongation onwards, which coincided with increase in aphid density, the index decreased to between 0.0 and 0.5 . Patchiness changed with density over time (Figure 4). The spatial pattern was aggregated in the beginning of plant growing season, when the aphid density was

Table 6. Parameters from Taylor's Power Law regression for the cabbage aphid, Brevicoryne brassicae on canola, Isfahan, Iran, 2011-2012.

| $\mathrm{PGP}^{a}$ | Aphid <br> group | df | $\mathrm{F}^{b}$ | $\mathrm{r}^{2}(\mathrm{adj})$ | $\mathrm{a} \pm \mathrm{SE}$ | $\mathrm{b} \pm \mathrm{SE}$ | $\mathrm{t}_{\text {cal }}{ }^{c}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PGP1 | Apterous | 20 | $111.53^{* *}$ | 0.85 | $0.65 \pm 0.35$ | $1.26 \pm 0.12$ | $2.19^{*}$ |
|  | Alates | 20 | $22.15^{* *}$ | 0.51 | $0.14 \pm 0.52$ | $1.29 \pm 0.27$ | 1.07 |
|  | Total | 20 | $71.91^{* *}$ | 0.78 | $0.07 \pm 0.49$ | $1.44 \pm 0.17$ | $2.59^{*}$ |
| PGP2 | Apterous | 10 | $135.58^{* *}$ | 0.93 | $0.41 \pm 0.41$ | $1.26 \pm 0.11$ | $2.40^{*}$ |
|  | Alates | 10 | $23.86^{* *}$ | 0.73 | $-0.45 \pm 0.80$ | $1.55 \pm 0.32$ | 1.74 |
|  | Total | 10 | $158.86^{* *}$ | 0.95 | $0.046 \pm 0.01$ | $1.34 \pm 0.11$ | $3.24^{*}$ |

${ }^{\text {a }}$ Plant Growth Phases, PGP1: From plant emergence to the end of rosette and PGP2: From the beginning of stem elongation to ripening; ${ }^{\text {b }}$ Significant at $P<0.01(* *)$ for $H_{0}: b=0,{ }^{c}$ Significant $P<$ $0.05\left(^{*}\right)$ for $H_{0}: b=1, t_{c a l}=(b-1) / S E_{b}, d f=n-1$ (Feng and Nowierski, 1992).
Table 7. Parameters from Iwao's Patchiness Regression for the cabbage aphid, Brevicoryne brassicae on canola, Isfahan, Iran, 2011-12.

| PGP $^{a}$ | Aphid <br> group | df | $\mathrm{F}^{b}$ | $\mathrm{r}^{2}(\mathrm{adj})$ | $\alpha \pm \mathrm{SE}$ | $\mathrm{t}_{\text {cal }}$ for <br> $\alpha^{c}$ | $\beta \pm \mathrm{SE}$ | $\mathrm{t}_{\text {cal }}$ for <br> $\beta^{d}$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PGP1 | Apterous | 20 | $43536.4^{* *}$ | 0.99 | $21.23 \pm 12.56$ | 1.69 | $1.01 \pm 0.00$ | $2.3^{*}$ |
|  | Alates | 20 | $25397^{* *}$ | 0.99 | $0.87 \pm 1.92$ | 0.45 | $1.05 \pm 0.02$ | $2.38^{*}$ |
|  | Total | 20 | $45711.3^{* *}$ | 0.99 | $16.70 \pm 12.59$ | 1.33 | $1.01 \pm 0.00$ | $2.58^{*}$ |
| PGP2 | Apterous | 10 | $471644^{* *}$ | 0.99 | $16.09 \pm 7.09$ | $2.27^{*}$ | $1.00 \pm 0.00$ | 2.17 |
|  | Alates | 10 | $15211.2^{* *}$ | 0.99 | $0.85 \pm 3.98$ | 0.21 | $1.02 \pm 0.01$ | $3.06^{*}$ |
|  | Total | 10 | $4715933^{* *}$ | 0.99 | $13.35 \pm 7.31$ | 1.83 | $1.00 \pm 0.00$ | $2.75^{*}$ |

[^3]


Figure 3. Temporal changes in the estimates of the aggregation index ( $1 / \mathrm{k}$ ) in relation to mean density (m) of the cabbage aphid, Brevicoryne brassicae on canola, Isfahan, Iran, 2011-2012. It was calculated by the formula of $1 / k=(m * / m)-1$, where: $1 / k$ is aggregation index or Cassie's index C and $(m * / m)$ is Lloyd's patchiness index. The values of $1 / k<$, $=$, and $>0$ represent regularity, randomness, and aggregation of the population in spatial pattern, respectively (Feng and Nowierski, 1992).


Figure 4. Within-field distribution of the cabbage aphid, Brevicoryne brassicae during growth season on canola, Isfahan, Iran, 2011-2012. Lloyd's Patchiness Index was calculated as $m^{*} / m$, where $m^{*}$ is Lloyd's mean crowding index and calculated as $m^{*}=m+\left(\left(s^{2} / m\right)-1\right)$, in which $m$ is mean and $s^{2}$ is variance of mean (Lloyd, 1967).
low. As the aphid population increased during the season, the size of aggregate was gradually reduced. The fit of a $k_{c}$ is justified only for apterous and total population in PGP2, with the values of 563.01 and 570.36, respectively. Figure 5 shows the optimum sample size required to estimate the mean of the total population of the aphid within $10 \%$ precision. At low densities, larger sample size is required. However, with increasing the mean density, the required sample size decreased rapidly.

## DISCUSSION

Among several aphids infesting different brassica crops, generally cabbage aphid was the most prevalent during the growing season (e.g. Trumble, 1982; Raworth et al., 1984). The sampling procedure was unbiased because the aphid lived exclusively on plant tissues and, in any block, the chances of selecting one plant in a sample was equal. Results showed that methods of heat-extracting and volumetric sub-sampling used on the kale, Brassica oleracea L. (Rawoth et al., 1984), were also good for the aphid estimation on canola. The results of

NANOVA suggested that substantial gains in precision could be realized by selecting the proper size of sampling unit. Therefore, a random sampling procedure could provide accurate estimate of the cabbage aphid population density in canola fields.

It seems that during the growth of stems and initiation of inflorescence, mature leaves of canola become unsuitable for the aphid development and it tended to aggregate on newly formed stems, leading to the highest aphid density in stem elongation stage. Trumble (1982) reported that the cabbage aphid occurred primarily on the highest and youngest leaves and readily migrated into the developing heads on Broccoli. These findings stressed on the role of host plant phenology in the population dynamics of the aphid. Sampling only upper part (10-15 cm upper part of plant's stems) may underestimate the aphid population density on canola, and is unsuitable for absolute estimation of aphid density. The tendency of the aphid to exhibit settling or feeding preference for the upper part necessitates the inspection of both stem parts when sampling for the cabbage aphid.

Non-uniform vertical distribution of aphids on the host plants is also reported for


Figure 5. Relationship between optimum sample size (number of canola plant) and mean population density (number of total cabbage aphid/plant), for $10 \%$ level of precision. It was calculated by the formula of $n=a\left(t_{\alpha / 2} / d\right)^{2}\left(m^{b-2}\right)$, where ' $a$ ' and ' $b$ ' are Taylor's coefficients, $t_{\alpha / 2}$ is the upper ${ }_{\alpha / 2}$ part of the standard normal distribution (for large samples $\mathrm{t}_{\alpha / 2}=1.96$ ), d is fixed proportion of the mean and m is sample mean (Buntin, 1994).
other aphid species (Jansson and Smilowitz, 1985; McCornack et al., 2008). This vertical distribution may be related to plant phenology (Jansson and Smilowitz, 1985). The factors that influence preference of the cabbage aphid on canola plant parts are not known. This phenomenon may be partly because this aphid, like the soybean aphid, Aphis glycines Matsumura, colonized the newly developed plant parts (i.e. upper plant parts) at first (McCornack et al., 2008). Mean demand, which is a concept related to Lloyd's mean crowding, represents the density of individuals (including self) per sampling unit that a typical individual can expect (Hartley and Shorrocks, 2002). Both of these can be used more generally as a measure of aggregation in the population, however, mean demand is often a more relevant measure than straightforward mean (Hartely and Shorrocks, 2002). The large values of mean demand indicate strong intraspecific interactions among individuals of the aphid, especially in stem elongation stage.

In general, distribution patterns of arthropods populations, including aphids, in agro- ecosystems commonly exhibit some degree of aggregation (Taylor, 1984). Regarding several non-regression indices, such as $s^{2} / m, I_{\delta}$, and $k$, population of other aphids, including Aphis gossypii Glover on cotton, showed aggregated distribution (Afshari et al., 2009). Departure from aggregation for $4 \%$ of the data set could be related to the change from aggregated to random distribution on the $6^{\text {th }}$ data set, which may be the result of unusual low total population density on this date. The result was in accordance with Myers (1978), who stated that Green's index and standardized Morisita's index were not influenced by population density and are good measures for describing spatial distribution.

TPL regression showed that alates had a random distribution. The same result was reported by Afshari and Dastranj (2010) for alates of cereal aphids. The good fit of

TPL to the data, is also confirmed by comparing the calculated coefficients to the typical range of the Taylor's coefficients, in which coefficient ' $a$ ' values ranged from 1 to 20 for highly aggregated species, and coefficient ' $b$ ' values ranged from 1 to 1.6 (Wilson, 1994). In the present study, the spatial pattern of alates and apterous when using non-regression indices was similar, but their patterns were different when using regression indices. The similar results were found for different growth stages of Hypera postica (Gyllenhal) (MoradiVajargah et al., 2011). Based on homogeneity tests TPL parameters are independent of the plant growth stage. Homogeneity of the spatial pattern throughout the growth season was also reported for other aphids like Sitobion avenae (F.) (Ward et al., 1986). In present study, IPR regression had high values of $r^{2}$. However, the range of means is very high and based on Davis (1994) it seems that IPR provides biased estimates. In general, among different studied spatial distribution indices, TPL could describe well the relationship between variance and mean of the data sets. Suitability of TPL over IPR is reported for other aphids (Elliot and Kieckhefer, 1986; Celini and Vaillant, 2004), and also for some mites (Darbemamieh et al., 2009; Khodayari et al., 2010; Rahmani et al., 2010).

The general trend of aphid distribution during the growth season in the field has been reported as follows: during the initial phase of infestation, it is random and becomes contagious as each aphid reproduces and increases its population (Southwood and Henderson, 2000). Temporal trend of spatial dispersion of the cabbage aphid in the present study is different from the abovementioned general trend, and is the same as reported by Badenhausser (1994) for Brachycaudus helichrysi Kalt. It seems that the crop is colonized by alates, which do not always have random distribution. Aphid colonies built up around the successfully
established alate landings, and this resulted in aggregated distribution for apterous, even at the beginning of infestation when the overall densities were low.
A common $k$ could not be fitted to all data, which might be because of the large variation in densities. In general, because ' $k$ ' changes dynamically with density, derivation of a common $k$ makes little biological or statistical sense, except possibly when the negative binomial distribution is to be applied over a limited range of densities (Wilson, 1994). The specific sample size used in this study ( 20 plants/field) was rather large to estimate the mean under a very sensitive precision level of $10 \%$, especially at high population densities.
The aim of the present study was to estimate the number of the cabbage aphid on an absolute basis (density per canola plant). Since the results of this type of sampling may be used to derive functional relationships, a greater number of sampling units may have to be examined to achieve sufficient reliability in the estimate (Wilson, 1994). This sampling procedure, however, is labor intensive and time-consuming and would not be suitable for monitoring programs. Although an accurate sampling method is not necessary for commercial monitoring or implementing control tactics, development of a reliable monitoring program depends on adequate knowledge of the target pest, including within- and between-plant distribution patterns and factors responsible for the observed patterns. The present study provides this type of necessary knowledge for the cabbage aphid on canola and will be used to develop an efficient monitoring scheme.

## ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Tarbiat Modares University for financial support and Isfahan Research

Center for Agriculture and Natural Resources for technical support.

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# رويه نمونهبردارى و توزيع زمانى- مكانى شته مومى كلم 

## (Brevicoryne brassicae)

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## چكيده


#### Abstract

تراكم جمعيت شته مومى كلم، (.Brevicoryne brassicae (L)، در دو مزرعه كلزاى سمياشى نشده در اصفهان طى سالهماى  بردارى در دو فاز رشدى گیاه (فاز اول: از زمان سبز شدن تا پاياين رزت، و واز دوم: از آغاز ساقهدهى تا تا   (بقيه طول ساقه) با استفاده از آزمون تى مقايسه شد. تغييرات زمانى در توزيع فضايى طى فصل رشا رشد با استفاده از شاخصهاى 1/k و بر اكند گى لويد ارزيابى شد. نتايج نشان داد كه بيشتر تغييرات كل مشاهـهـ      


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[^1]:    * Subsamples in which stage/morph distributions differed from stage/morph distribution of the main sample, using Chi-square test at $P<0.05$. Different letters in each sub-sample show which stage/morph category in the sub-sample differed from stage/morph category in the main sample, multiple comparisons using Bonferroni Chi-square test at $P<0.05$.

[^2]:    ${ }^{a}$ Plant Growth Phases, PGP1: From plant emergence to the end of rosette and PGP2: From the beginning of stem elongation to ripening.
    ${ }^{b}$ Refer to data set, which is sampling date $\times$ field.

[^3]:    ${ }^{a}$ Plant Growth Phases, PGP1: From plant emergence to the end of rosette and PGP2: From the beginning of stem elongation to ripening; ${ }^{b}$ Significant at $P<0.01(* *)$ for $H_{0}: b=0 ;{ }^{c}$ Significant at $\mathrm{P}<0.05\left(^{*}\right)$ for $H_{0}: \alpha=0, t_{c a l}=\alpha / S E_{\alpha}, d f=n-1$ (Davis, 1994), ${ }^{d}$ Significant at $\mathrm{P}<0.05\left(^{*}\right)$ for $H_{0}: \beta=1$, $t_{c a l}=(\beta-1) / S E_{\beta}, d f=n-1$ (Feng and Nowierski, 1992).

