Population Variation of a Specialist versus a Generalist Aphid Sharing the Same Host Plant in Field

M. R. Nematollahi¹, Y. Fathipour¹*, A. A. Talebi¹, J. Karimzadeh², and M. P. Zalucki³

ABSTRACT

Aphids in unsprayed canola (Brassica napus Linnaeus) fields in Isfahan province (central Iran) were sampled for two growing seasons, 2011-2013. Sampling unit was a whole plant and 20 plants were sampled weekly. In the laboratory, heat-extraction and sub-sampling techniques were used to estimate the density of aphids. To determine the relationship between population growth rate of the aphids and degree-days, linear regressions were done between log of aphid density and accumulated degree-days. Homogeneity tests were done using pairwise comparisons between slopes. Aphids' preference for upper (10-15 cm upper part of stem) and lower (the rest of stem) parts of the plant was analyzed using Student’s t-test. Aphid fauna included: Cabbage Aphid [CA; Brevicoryne brassicae (L.)], Green Peach Aphid [GPA; Myzus persicae (Sulzer)], and Turnip Aphid [TA; Lipaphis erysimi (Kaltenbach)]. The GPA developed small population in comparison with CA and TA and was occasionally found. The population density of CA and GPA tended to show two peaks, and during flower initiation, population began to decrease. The average population growth rates of GPA and CA were 0.001 and 0.003, respectively. Homogeneity tests indicated that, at all sites and years, GPA showed reduced growth rate compared to CA. On average, 36 and 64% of CA and GPA populations were found on the lower parts of plants, respectively. This indicated that CA preferred upper part while GPA preferred lower part of the plants. The obtained results could be used to make a contribution to systematize the field monitoring of predominant aphids in canola crop.

Keywords: Brevicoryne brassicae, Canola, Degree-days, Myzus persicae, Population growth rate.

INTRODUCTION

The arthropod fauna associated with Brassica spp. is extensive, with 150 or more species in 25 or more families considered being major or minor pests of brassicaceous crops (Bonnemaison, 1965; Boyd and Lentz, 1994). Four aphid species, namely, Cabbage Aphid (CA; Brevicoryne brassicae (Linnaeus)), Green Peach Aphid (GPA; Myzus persicae (Sulzer)), Turnip Aphid (TA; Lipaphis erysimi (Kaltenbach)) and cotton aphid (Aphis gossypii Glover) infest canola plants (Brassica napus Linnaeus) (Lamb, 1989), but details on the fauna differ depending on location. For example, in Tennessee, three species of aphids, TA, GPA, and CA were found, with TA as the most abundant species (Boyd and Lentz, 1994). In France, GPA is one of the most serious pests that attack canola in autumn; CA and TA were not known to be a problem in canola in autumn (Desneux et al., 2006). In Iran, canola is one of the most important oilseed crops, with more than 86,000 ha under cultivation and yield of nearly 2,080

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kg ha$^{-1}$ (Anonymous, 2010). Aphid fauna associated with canola production was studied in some parts of Iran. In Sistan, south-eastern Iran, four species of aphids, namely, GPA, CA, cotton aphid and *Acyrthosiphon gossypii* Mordvilko were found, with CA as the dominant species (Modarres-Najafabadi et al., 2004). Studies in Khuzestan, southern Iran, revealed that three species of aphids i.e. TA, CA and GPA, attack canola plants and TA was the dominant species (Khajehzadeh et al., 2010; Farsi et al., 2009).

Relationship between population growth rate and degree-days was studied in some aphid species (e.g., Wright et al., 1995; Jansson and Smilowitz, 1985). Aphid responses to temperature are similar to those of other insects. Most aphid species show a strong linear relationship between temperature and growth or development within a range of approximately 7 and 25°C, followed by a decline at increasing temperatures (Awmack and Leather, 2007). Describing the within-plant distribution is critical to the development of sampling plans, particularly for aphids (McCornack et al., 2008). Aphids often feed preferentially on certain parts of a plant, and some parts may be more susceptible to damage (Wratten et al., 2007). CA significantly preferred the upper (10-15 cm upper part of stem) to lower (the rest of stem) part of canola plants (Nematollahi et al., 2014a, b). In contrast, it has been shown that GPA had a distinct preference for older, lower leaves on some plants (Jansson and Smilowitz, 1985). In general, alate production is stimulated by crowding, although the proportion of the population developing into alate may vary among populations of the same aphid species on different host plant species (Williams and Dixon, 2007). However, not all aphid species respond in this manner. For example, experiment with GPA showed that this species does not always increase production of winged morphs in response to crowding (Williams et al., 2000).

The aims of this study were: (1) To identify aphids associated with canola plantings in unsprayed canola fields during two growing season, from 2011 to 2013; (2) To compare the seasonal occurrence and abundance of two abundant aphid species, and (3) To assess density-dependence of alate production for CA.

**MATERIALS AND METHODS**

**The Study Site and Sampling Procedure**

Field experiments were conducted at two sites in Isfahan province, central Iran (Site 1: Isfahan 32° 30’ 34” N and 51° 49’ 57” E at 1,547 m altitude, and Site 2: Alavije 33° 04’ 56” N and 51° 11’ 08” E at 1,814 m altitude) for two growing seasons, 2011-2013. In each site, canola cv. Okapi (the most common cultivar grown in Isfahan province) was planted into two fields (each 500 m²). Sampling unit was a whole plant and 20 plants were sampled weekly during a 32-week period, from plant emergence (mid-October) to crop harvest (late May). No insecticide was applied on or around the experimental fields. Population density of aphids was recorded in two Plant Growth Phases (PGP): from plant emergence to the end of rosette (PGP1), and from the beginning of stem elongation to ripening (PGP2). The average plant growth stages of the crop were recorded using the key provided by Harper and Berkenkamp (1975) with minor modifications, thus, allowing stem elongation instead of bud. Sampling involved uprooting or cutting plants at ground level and placing them individually into plastic bags. In the laboratory, heat-extraction and sub-sampling methods were used to estimate the number of aphids in each sample, as described in Nematollahi et al. (2014b). Before heat-extraction, adult alate aphids and adult parasitoid and hyperparasitoid wasps were collected. The aphids in the samples were separated by species and counted. The seasonal trends of the population density of the aphids were
plotted at each site. Data were normalized using the $\log(x+1)$ transformation. The effect of sampling date on aphid density was tested with repeated measures mixed model ANOVA, where date repeated within location was considered as a random effect (SAS Institute, 2004).

**Relationship between Population Growth Rate and Degree-Days**

Sampling weeks were converted to Degree-Days ($DD$) using the half-day sine wave method (Higley et al., 1986), using the following formula:

$$DD = \left(\frac{T_{\text{max}}+T_{\text{min}}}{2}\right) + \left(\frac{T_{\text{max}}-T_{\text{min}}}{2}\right)\sin(-1.5708 + 0.2618 j)$$

Where, $T_{\text{max}}$ is the maximum daily temperature, $T_{\text{min}}$ is the minimum daily temperature and $j$ is the numbers of hours pass the minimum for that day. Temperature data was obtained from weather stations located less than 2 km from experimental sites. Accumulated degree-days over the first 12 hours were calculated from that day’s minimum and maximum air temperatures, and for the next 12 hours were calculated from that day’s maximum temperature and the next day’s minimum temperature (Young and Young, 1998). The base temperature of 5°C (Campbell et al., 1974) and 4°C (Ro et al., 1998) were used for CA and GPA, respectively. Degree-days were accumulated for each site from mid-October, i.e., approximate date of plant emergence. To determine the relationship between degree-days and population growth rate of aphids, linear regressions were done for each site and year using degree-days as the independent variable and the log mean number of each aphid per plant as the dependent variable (Wright et al., 1995). The slope ($b$) of each regression was used as a measure of the population growth rate. The homogeneity tests were used for pairwise comparison between slopes (Feng and Nowierski, 1992), using student’s $t$-test formula:

$$t = \frac{(b_1-b_2)/(SE_1^2+SE_2^2)^{1/2}}$$

Where, $df= n_1+n_2-2$, 1 and 2 are pairs, respectively.

**Within-plant Distribution and Density-dependence of Alate Production**

Within-plant distribution of aphids was most obvious on taller plants where physiological differences between leaves and/or plant parts were greater (Trumble, 1982b). To check distribution of the aphids within a canola plant, aphid densities in PGP2 on each plant was recorded in upper (10-15 cm upper part of stem) and lower (the rest of stem) parts, and aphid preference for these parts was analyzed using Student’s $t$-test (SAS Institute, 2004). To check if production of CA alates was related to aphid density, a linear regression of alates number (dependent variable) on the total population density of the aphid per plant (independent variable) was done (Raworth et al., 1984). Significant regressions with positive slopes indicated that the production of alates increased with increased aphid density, which was interpreted as evidence of density-dependence (Wright et al., 1995).

**RESULTS**

**Aphid Fauna And Their Seasonal Trends**

During 2011-2012 growing season, two aphid species i.e., CA and GPA were collected, and during 2012-2013, an additional species, TA was collected. During this study, TA was collected occasionally on some dates
and, therefore, not included in our analyses. The CA was the most abundant species collected and accounted for 98% of the aphids sampled. We observed that CA feeding lead to stunted growth, decreased pod and seed production, especially at stem elongation and flowering stages. The GPA developed relatively small population in comparison to CA (409,215 GPA compared to 20,052,562 CA); however it was the primary contaminant of canola plants during rosette and stem elongation stages (Figure 2). Repeated measures ANOVA showed that in all sites and years the population density of both aphids varied with sampling week (See statistics in Figure 1 caption). The time of peak density of CA varied among different sites and years; however, the peaks were more pronounced at Isfahan than at Alavije. In general, the population of both aphids tended to show two peaks. There was an initial rise in aphids’ number, leading to a small peak at the rosette stage. As the season progressed, the population densities increased to a much higher peak before declining once more. For CA, this second peak or seasonal peak usually occurred 2-3 weeks later than that for GPA (Figure 1).

In general, as plants started to flower, population of both aphids began to decrease, except for GPA at one site (Figure 1-c). At this site, when flowering was completed (27th week of sampling) and plants began to ripen, CA densities declined rapidly, while density of GPA rose thereafter.

**Relationship between Population Growth Rate and Degree-days**

The regression of log mean number of aphids per plant on accumulated degree-days was highly significant. Population growth

![Figure 1](image-url)
Aphids Populations in Canola Field

Figure 2. Population density of cabbage aphid and green peach aphid in different canola growth stages: (a) Isfahan, 2011-2012; (b) Isfahan, 2012-2013; (c) Alavije, 2011-2012, and (d) Alavije, 2012-2013. Vertical bars represent the standard errors of the means.

Table 1. Parameters from linear regression of the logarithm of population density of Cabbage Aphid (CA) and Green Peach Aphid (GPA) on accumulated degree-days on canola.

<table>
<thead>
<tr>
<th>Site</th>
<th>Insect</th>
<th>df</th>
<th>Year</th>
<th>$F^c$</th>
<th>$r_{adj}^b$</th>
<th>$Y_{intercept}$ (±SE)</th>
<th>Slope (b±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isfahan CA</td>
<td>1.21</td>
<td>2011-12</td>
<td>222.97**</td>
<td>0.90</td>
<td>-0.69±0.25</td>
<td>0.004±0.0003</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.24</td>
<td>2012-13</td>
<td>64.12**</td>
<td>0.71</td>
<td>0.39±0.29</td>
<td>0.003±0.0004</td>
<td></td>
</tr>
<tr>
<td>GPA</td>
<td>1.18</td>
<td>2011-12</td>
<td>94.87**</td>
<td>0.83</td>
<td>-1.37±0.24</td>
<td>0.002±0.0002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.21</td>
<td>2012-13</td>
<td>35.45**</td>
<td>0.61</td>
<td>0.05±0.21</td>
<td>0.001±0.0003</td>
<td></td>
</tr>
<tr>
<td>Alavije CA</td>
<td>1.23</td>
<td>2011-12</td>
<td>274.31**</td>
<td>0.91</td>
<td>-0.24±0.17</td>
<td>0.003±0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>2012-13</td>
<td>110.54**</td>
<td>0.80</td>
<td>0.08±0.21</td>
<td>0.002±0.0002</td>
<td></td>
</tr>
<tr>
<td>GPA</td>
<td>1.21</td>
<td>2011-12</td>
<td>211.80**</td>
<td>0.90</td>
<td>-0.26±0.28</td>
<td>0.002±0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.21</td>
<td>2012-13</td>
<td>14.15**</td>
<td>0.37</td>
<td>-0.32±0.05</td>
<td>0.001±0.0003</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Data for each site in each growing season was average of two fields. $^b$ Significant at the level of $P<0.01$.

rates [Slope(b)] ranged from 0.002 to 0.004 for CA and from 0.001 to 0.002 for GPA (Table 1). In most cases, pairwise comparisons of slopes for each aphid between years and within site were significant, except during 2011-2012 where such significance was not observed when GPA at Isfahan was compared to GPA at Alavije (Table 2). The average temperature and relative humidity for each site, during 32-week sampling period, were as follow; Alavijeh, 2011-2012: 12.3°C±0.45, 36.7%±0.85; Alavijeh, 2012-2013: 10.6°C±0.44, 45.6%±0.79; Isfahan, 2011-2012: 11.7°C±0.45, 40.3%±0.68; Isfahan, 2012-2013: 10.3°C±0.44, 46.4%±1.12.

Within-plant Distribution and Density-dependence of Alate Production

Data collected from the beginning of stem elongation to ripening (PGP2) showed that 36 and 64% of the total population of CA and GPA were found on the lower parts of
Table 2. Homogeneity tests\textsuperscript{a} for slopes from regression of the logarithm of population density of Cabbage Aphid (CA) and Green Peach Aphid (GPA) on accumulated degree-days on canola.

<table>
<thead>
<tr>
<th>Pairs\textsuperscript{b} (1 vs. 2)</th>
<th>$b_1$</th>
<th>$b_2$</th>
<th>$SE_1$</th>
<th>$SE_2$</th>
<th>df</th>
<th>$t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A vs. B</td>
<td>0.0049</td>
<td>0.0035</td>
<td>0.0003</td>
<td>0.0004</td>
<td>47</td>
<td>2.80**</td>
</tr>
<tr>
<td>A vs. C</td>
<td>0.0049</td>
<td>0.0033</td>
<td>0.0003</td>
<td>0.0002</td>
<td>46</td>
<td>4.43**</td>
</tr>
<tr>
<td>B vs. D</td>
<td>0.0035</td>
<td>0.0025</td>
<td>0.0004</td>
<td>0.0002</td>
<td>50</td>
<td>2.80**</td>
</tr>
<tr>
<td>C vs. D</td>
<td>0.0033</td>
<td>0.0025</td>
<td>0.0002</td>
<td>0.0002</td>
<td>51</td>
<td>2.23**</td>
</tr>
<tr>
<td>E vs. F</td>
<td>0.0029</td>
<td>0.0018</td>
<td>0.0003</td>
<td>0.0003</td>
<td>41</td>
<td>2.59**</td>
</tr>
<tr>
<td>E vs. G</td>
<td>0.0029</td>
<td>0.0023</td>
<td>0.0003</td>
<td>0.0001</td>
<td>41</td>
<td>1.89</td>
</tr>
<tr>
<td>F vs. H</td>
<td>0.0018</td>
<td>0.0012</td>
<td>0.0003</td>
<td>0.0003</td>
<td>50</td>
<td>3.47**</td>
</tr>
<tr>
<td>G vs. H</td>
<td>0.0023</td>
<td>0.0012</td>
<td>0.0001</td>
<td>0.0003</td>
<td>51</td>
<td>1.41**</td>
</tr>
<tr>
<td>A vs. E</td>
<td>0.0049</td>
<td>0.0029</td>
<td>0.0003</td>
<td>0.0003</td>
<td>41</td>
<td>4.71**</td>
</tr>
<tr>
<td>B vs. F</td>
<td>0.0035</td>
<td>0.0018</td>
<td>0.0004</td>
<td>0.0003</td>
<td>47</td>
<td>3.60**</td>
</tr>
<tr>
<td>C vs. G</td>
<td>0.0033</td>
<td>0.0023</td>
<td>0.0002</td>
<td>0.0001</td>
<td>46</td>
<td>4.47**</td>
</tr>
<tr>
<td>D vs. H</td>
<td>0.0025</td>
<td>0.0012</td>
<td>0.0002</td>
<td>0.0003</td>
<td>48</td>
<td>3.60**</td>
</tr>
</tbody>
</table>

\textsuperscript{a} $H_0$: $b_1 = b_2$. \textsuperscript{b} (A) CA, Isfahan, 2011-2012; (B) CA, Isfahan, 2012-2013; (C) CA, Alavije, 2011-2012; (D) CA, Alavije, 2012-2013; (E) GPA, Isfahan, 2011-2012; (F) GPA, Isfahan, 2012-2013; (G) GPA, Alavije, 2011-2012, and (H) GPA, Alavije, 2012-2013. \textsuperscript{c} Table $t$'s were estimated using linear interpolation. ** Pairs are significant at the level of P< 0.01.

Table 3. Within-plant distribution of Cabbage Aphid (CA) and Green Peach Aphid (GPA) on canola in PGP2\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Site\textsuperscript{b}</th>
<th>Insect</th>
<th>Year</th>
<th>Aphids/ plant (Mean± SE)</th>
<th>df</th>
<th>pr &gt; t\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower part of plant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isfahan CA</td>
<td>CA</td>
<td>2011-12</td>
<td>3974.8± 204.76</td>
<td>7714.8± 22.94</td>
<td>439</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2012-13</td>
<td>1767.6± 40.22</td>
<td>3835.6± 138.9</td>
<td>439</td>
</tr>
<tr>
<td>GPA</td>
<td></td>
<td>2011-12</td>
<td>4.43± 0.26</td>
<td>2.29± 0.15</td>
<td>439</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2012-13</td>
<td>9.11± 0.47</td>
<td>5.11± 0.32</td>
<td>439</td>
</tr>
<tr>
<td>Alavije CA</td>
<td>CA</td>
<td>2011-12</td>
<td>2415.5± 77.97</td>
<td>4991.3± 146.38</td>
<td>439</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2012-13</td>
<td>808.34± 29.81</td>
<td>1301.4± 44.66</td>
<td>439</td>
</tr>
<tr>
<td>GPA</td>
<td></td>
<td>2011-12</td>
<td>10.1± 0.34</td>
<td>4.50± 0.11</td>
<td>439</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2012-13</td>
<td>5.40± 0.20</td>
<td>3.02± 0.14</td>
<td>439</td>
</tr>
</tbody>
</table>

\textsuperscript{a} From the beginning of stem elongation to ripening. \textsuperscript{b} Data for each site in each growing season was average of two fields. \textsuperscript{c} Significant differences between means (unequal variances), using paired Student’s $t$-test at $P<0.01$. plants, respectively (Table 3). This indicated that CA preferred the upper parts, while GPA populations, on the contrary, preferred the lower parts of the plants. Regressions of alates number against the total population density were significant with positive slopes in all experimental fields (Table 4).

**DISCUSSION**

Generally, once flowering was completed, GPA population density began to decrease possibly because green foliage became less available. However, at Alavije in 2012-2013 growing season, a different trend was observed because the site was near an orchard, and, therefore, may account for the increasing density of GPA which is a generalist species that could have continued to feed on plants around the orchard well after canola plants had completed flowering.

Aphid growth and developmental rates have been used extensively to predict the performance of aphid populations on plants because they correlate well with fecundity and the intrinsic rate of increase ($r_m$) (Leather and Dixon, 1984; Awmack and...
Table 4. Parameters from linear regression of alate number on total population density for cabbage aphid on canola.

<table>
<thead>
<tr>
<th>Site</th>
<th>df</th>
<th>Year</th>
<th>$F^b$</th>
<th>$r_{adj}^2$</th>
<th>$Y_{intercept}$ (a±SE)</th>
<th>Slope (b±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isfahan 1, 30</td>
<td>2011-12</td>
<td>450.44**</td>
<td>0.93</td>
<td>53.29± 11.90</td>
<td>0.026± 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2012-13</td>
<td>101.88**</td>
<td>0.76</td>
<td>5.20± 31.75</td>
<td>0.082± 0.008</td>
<td></td>
</tr>
<tr>
<td>Alavije 1, 30</td>
<td>2011-12</td>
<td>211.22**</td>
<td>0.87</td>
<td>5.49± 14.39</td>
<td>0.042± 0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2012-13</td>
<td>180.90**</td>
<td>0.85</td>
<td>-2.76± 11.88</td>
<td>0.105± 0.007</td>
<td></td>
</tr>
</tbody>
</table>

*a Data for each site in each growing season was average of two fields. *b Significant at the level of $P<0.01$.

Leather, 2007). Rates of population increase in the present study ranged from 0.001 to 0.004, and were similar to that obtained by Wright et al. (1995) for hop aphid, *Phorodon humuli* (Scherank) on *Prunus* spp. Pairwise comparisons of slopes between years and between sites indicated that in each site within a year, population growth rate of both aphids was closely related to accumulated degree-days, but within a site, the rate varied among years. The same result was obtained by Wright et al. (1995) for hop aphid. Homogeneity tests showed that in all sites and years, GPA grew more slowly with respect to degree-days than CA. Even though many biotic and abiotic factors regulate physiological processes of aphid development (Lees, 1966), the most important single factor for the development of aphids is often temperature (Campbell et al., 1974; Ro et al., 1998). Temperature may affect greatly aphid growth and developmental rates. For example, aphids reared at high temperature may grow into small adults containing fewer embryos (Leather and Dixon, 1982; Collins and Leather, 2001).

Within-plant distribution indicated that CA preferred upper to lower plant parts and this preference was vice versa for GPA. Our results corroborate reports by van Emden and Bashford (1969) on the distribution of CA and GPA on Brussels sprouts (*Brassica oleracea* var. *gemmifera* Zenker). Preference of CA for younger parts and preference of GPA for older parts of plants has been reported on other *Brassica* crops (Trumble, 1982a, b; Dunn and Kempton, 1971; Hopkins et al., 1998). The vertical distribution of the aphids on canola may be particularly explained by differences in plant phenology and physiology along the vertical gradient of canola plants. It has been suggested that GPA require amino-nitrogen compounds mobilized during leaf senescence (van Emden, 1966). Thus, GPA would be expected to be more abundant on lower parts of plants.

Results showed that the production of CA alates on canola was density-dependent. Density dependence of production of alates of CA was reported on kale, *Brassica oleracea* var. *acephala* by Raworth et al. (1984), which suggested that increases in the production of the fourth instar alates could have resulted in large numbers of alates at high aphid densities. CA is a gregarious aphid (Hayamizu, 1982), therefore, as Müller et al. (2001) postulated, it is more responsive to the crowding stimulus than the non-gregarious species, like GPA.

The present study has shown that CA is a specialist on canola and prefers feeding on younger plant parts, which makes it of economic importance on canola as it can move into the developing floral buds and render it unmarketable. However, GPA is a generalist which feeds on a wide range of plants in several families, but is generally not of economic importance on canola. The obtained results could be used to make a contribution to systematize the field monitoring of predominant aphids in canola crop.
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نوسانات جمعیت یک شکه اختصاصی در مقابل یک شکه عمومی فعال روی یک میزان مشترک در مزرعه

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چکیده

شکه‌ها در مزارع سیب‌پایه نشده کلزا (Brassica napus L.) در استان اصفهان (مرکز ایران) تا طی دو فصل رشد، 1392-1390، میشوده و بوده و 40 گیاه به طور فضایی آزمایشگاهی از گیاهان گرده و نمونه‌برداری فرود گرفتند. نتایج نشان داد توجه بودن رشد جمعیت شکه‌ها استفاده شد. برای بررسی رابطه بین رشد جمعیت شکه‌ها و درجه-رود، برای روش تخمین جمعیت شکه‌ها استفاده شد. نتایج نشان داد که برای رشد جمعیت شکه‌ها، درجه-رود روش تخمین جمعیت به صورت مقایسه‌های جفتی بین شبیه‌ای انجام گرفت. نتایج نشان داد که برای بررسی سایه پایه‌ای نسبت به آزمون‌های نمونه‌گیری از آزمون تحلیل گردید. فرآیند نشان داد که برای بررسی سایه‌های نسبت به آزمون‌های نمونه‌گیری از آزمون تحلیل گردید. فرآیند نشان داد که برای بررسی سایه‌های نسبت به آزمون‌های نمونه‌گیری از آزمون تحلیل گردید. فرآیند
