

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

M. R. Nematollahi<sup>1</sup>, Y. Fathipour<sup>1\*</sup>, A. A. Talebi<sup>1</sup>, J. Karimzadeh<sup>2</sup>, and M. P. Zalucki<sup>3</sup>

### 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 (Bonnemaïson, 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

<sup>1</sup> Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

\* Corresponding author; e-mail: fathi@modares.ac.ir

<sup>2</sup> Department of Plant Protection, Isfahan Research Center for Agriculture and Natural Resources, P. O. Box: 81785-199, Isfahan, Islamic Republic of Iran.

<sup>3</sup> School of Biological Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia.



kg ha<sup>-1</sup> (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 *Acyrtosiphon 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 m<sup>2</sup>). 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:

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

Where,  $T_{\max}$  is the maximum daily temperature,  $T_{\min}$  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). Data from the start of sampling to the peak number of each aphid for each season were used in the regressions (Wright *et al.*, 1995; Jansson and Smilowitz, 1985). 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:

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

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

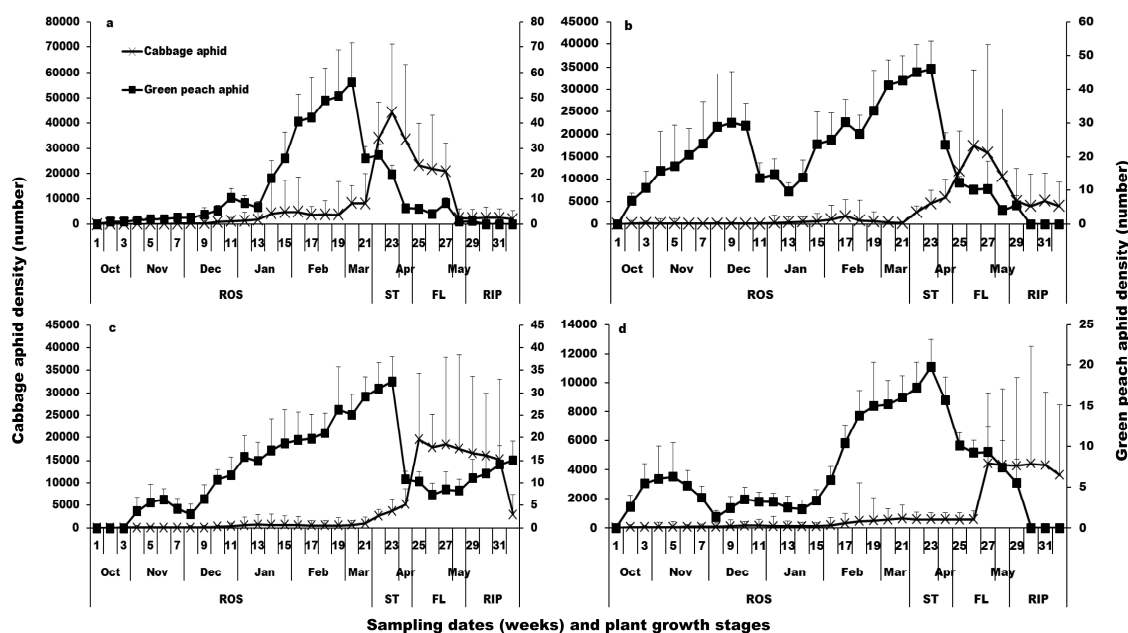


and, therefore, not included in our analyses. The CA was the most abundant species collected and accounted for 98% of the aphids sampled. We observed that CA feeding lead to stunted growth, decreased pod and seed production, especially at stem elongation and flowering stages. The GPA developed relatively 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 (27<sup>th</sup> 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

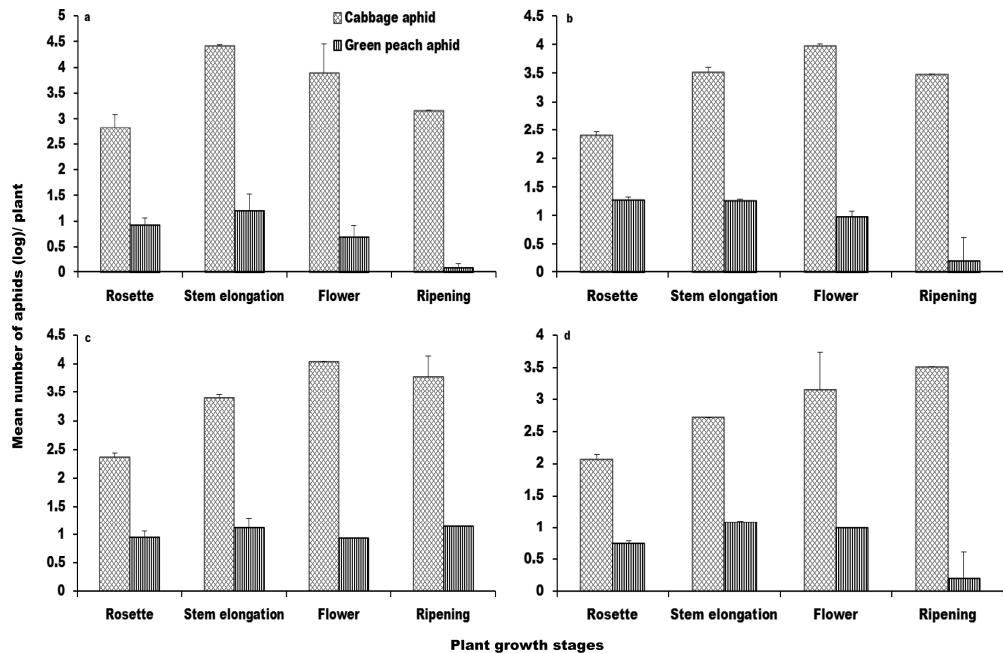
The regression of log mean number of aphids per plant on accumulated degree-days was highly significant. Population growth



**Figure 1.** Seasonal trends of population density of Cabbage Aphid (CA) and Green Peach Aphid (GPA) on canola: (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. Plant growth stages were as follows: ROS= Rosette, ST= Stem elongation, FL= Flowering, and RIP= Ripening.

Statistics of repeated measures ANOVA for CA were as follows: (a)  $F= 2,020.66$ ,  $df= 31, 1,209$ ,  $P < 0.0001$ ; (b)  $F= 1,880.51$ ,  $df= 31, 1,209$ ,  $P < 0.0001$ ; (c)  $F= 2,212.59$ ,  $df= 31, 1,209$ ,  $P < 0.000$ , and (d)  $F= 2,016.75$ ,  $df= 31, 1,209$ ,  $P < 0.0001$ .

Statistics of repeated measures ANOVA for GPA were as follows: (a)  $F= 1,528.51$ ,  $df= 31, 1,209$ ,  $P < 0.0001$ ; (b)  $F= 825.57$ ,  $df= 31, 1,209$ ,  $P < .0001$ ; (c)  $F= 781.98$ ,  $df= 31, 1,209$ ,  $P < 0.0001$ , and (d)  $F= 593.68$ ,  $df= 31, 1,209$ ,  $P < 0.0001$ .



**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.

Site <sup>a</sup>	Insect	df	Year	F <sup>b</sup>	r <sup>2</sup> <sub>adj</sub>	Y intercept (a±SE)	Slope (b±SE)
Isfahan	CA	1,21	2011-12	222.97**	0.90	-0.69± 0.25	0.004± 0.0003
		1,24	2012-13	64.12**	0.71	0.39± 0.29	0.003± 0.0004
	GPA	1,18	2011-12	94.87**	0.83	-1.37± 0.24	0.002± 0.0002
		1,21	2012-13	35.45**	0.61	0.05± 0.21	0.001± 0.0003
Alavije	CA	1,23	2011-12	274.31**	0.91	-0.24± 0.17	0.003± 0.0001
		1,25	2012-13	110.54**	0.80	0.08± 0.21	0.002± 0.0002
	GPA	1,21	2011-12	211.80**	0.90	-0.26± 0.28	0.002± 0.0001
		1,21	2012-13	14.15**	0.37	-0.32± 0.05	0.001± 0.0003

<sup>a</sup> Data for each site in each growing season was average of two fields. <sup>b</sup> 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<sup>a</sup> 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.

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

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

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 Alaviye 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 3.** Within-plant distribution of Cabbage Aphid (CA) and Green Peach Aphid (GPA) on canola in PGP2 <sup>a</sup>.

Site <sup>b</sup>	Insect	Year	Aphids/ plant (Mean± SE)		df	pr> t <sup>c</sup>
			Lower part of plant	Upper part of plant		
Isfahan	CA	2011-12	3974.8± 204.76	7714.8± 22.94	439	<0.0001**
		2012-13	1767.6± 40.22	3835.6± 138.9	439	<0.0001**
	GPA	2011-12	4.43± 0.26	2.29± 0.15	439	<0.0001**
		2012-13	9.11± 0.47	5.11± 0.32	439	<0.0001**
Alaviye	CA	2011-12	2415.5± 77.97	4991.3± 146.38	439	<0.0001**
		2012-13	808.34± 29.81	1301.4± 44.66	439	<0.0001**
	GPA	2011-12	10.1± 0.34	4.50± 0.11	439	<0.0001**
		2012-13	5.40± 0.20	3.02± 0.14	439	<0.0001**

<sup>a</sup> From the beginning of stem elongation to ripening, <sup>b</sup> Data for each site in each growing season was average of two fields. <sup>c</sup> Significant differences between means (unequal variances), using paired Student's  $t$ -test at  $P < 0.01$ .

**Table 4.** Parameters from linear regression of alate number on total population density for cabbage aphid on canola.

Site <sup>a</sup>	df	Year	F <sup>b</sup>	r <sup>2</sup> <sub>adj</sub>	Y intercept (a±SE)	Slope (b±SE)
Isfahan	1, 30	2011-12	450.44**	0.93	53.29± 11.90	0.026± 0.001
		2012-13	101.88**	0.76	5.20± 31.75	0.082± 0.008
Alavije	1, 30	2011-12	211.22**	0.87	5.49± 14.39	0.042± 0.002
		2012-13	180.90**	0.85	-2.76± 11.88	0.105± 0.007

<sup>a</sup> Data for each site in each growing season was average of two fields. <sup>b</sup> 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* L. 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 شته اختصاصی در مقابل یک شته عمومی فعال روی یک میزبان مشترک در مزرعه

م. ر. نعمت الهی، ی. فتحی پور، ع. ا. طالبی، ج. کریم زاده و م. پ. زالوکی

#### چکیده

شته‌ها در مزارع سم‌پاشی نشده کلزا (*Brassica napus* L.) در استان اصفهان (مرکز ایران) طی دو فصل رشد، ۱۳۹۲-۱۳۹۰ نمونه‌برداری شدند. واحد نمونه‌برداری یک گیاه کامل بود و ۲۰ گیاه به طور هفتگی نمونه‌برداری گردید. در آزمایشگاه، از تکنیک‌های جداسازی به روش گرمادهی و نمونه‌برداری فرعی برای تخمین جمعیت شته‌ها استفاده شد. برای تعیین رابطه بین نرخ رشد جمعیت شته‌ها و درجه-روز، رگرسیون خطی بین لگاریتم تراکم جمعیت شته و درجه-روز تجمعی برقرار شد. آزمون‌های همگنی به صورت مقایسه‌های جفتی بین شیب‌ها انجام گرفت. ترجیح شته‌ها برای بخش‌های بالایی (۱۰-۱۵ سانتی‌متر انتهایی ساقه) و پایینی (بقیه طول ساقه) گیاه با استفاده از آزمون تی تحلیل گردید. فون شته‌ها عبارت بود از: شته مومی کلم (*Brevicoryne brassicae* (L.))، شته سبز هلو (*Myzus persicae* (Sulzer))، و شته خردل (*Lipaphis erysimi* (Kaltenbach)). جمعیت شته سبز هلو نسبت به شته مومی کلم اندک بود و شته خردل به طور تصادفی جمع‌آوری شد. تراکم جمعیت شته‌های مومی کلم و سبز هلو دارای دو اوج بود و با آغاز گل‌دهی جمعیتشان رو به کاهش نهاد. متوسط نرخ رشد جمعیت برای شته‌های سبز هلو و مومی کلم به ترتیب ۰/۰۱ و ۰/۰۳ برآورد شد. آزمون‌های همگنی نشان داد که در همه سایت‌ها و سال‌ها، شته سبز هلو نسبت به شته مومی کلم نرخ رشد کمتری دارد. به طور متوسط به ترتیب ۳۶٪ و ۶۴٪ از جمعیت شته‌های مومی کلم و سبز هلو در بخش‌های پایینی گیاه یافت شدند و این نشان می‌دهد که شته مومی کلم بخش بالایی گیاه را ترجیح می‌دهد، در حالی که شته سبز هلو بخش پایینی گیاه را ترجیح می‌دهد. نتایج حاصله در راستای قاعده‌مند کردن پایش شته‌های غالب در مزارع کلزا قابل استفاده می‌باشد.