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Research Article

Population fluctuations of small walnut aphid *Chromaphis juglandicola* (Hemiptera: Aphididae) and its natural enemies in walnut orchards of northwestern Iran

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Abstract: Small walnut aphid (SWA) *Chromaphis juglandicola* is an economic pest of walnut trees. Population fluctuation of SWA was studied in Mamaghan walnut orchards (East Azarbaijan Province, Iran).Three factors affecting population density of SWA, including vertical divisions of canopy (upper and lower half), geographical orientations of canopy (at four levels) and elongation of branch (basal and distal ends), were investigated. Sampling unit was a cluster of five leaves. Totally 128 samples were taken weekly from all strata upon eight chosen trees, repeating 30 times during the season. Natural enemies also were counted. The first SWA individuals were observed early May, consisting of first instar larvae and alate females. A sudden population increase occurred in mid-May. Maximum 40 aphids/leaf were observed in early June with first and second instars dominant, followed by a sudden decline in late June. A small peak was observed at early October. A partial tendency was observed toward north of canopy, at basal half of downward branches. Positive linear correlation between natural enemies' and SWA populations suggests density dependence. Moreover 2-4 week delay was present between them. *Trioxys pallidus* (Holliday) was dominant natural enemy in the region. Contrary to previous works, overwintering stages of the SWA in the region were predominantly developed stages (third and fourth instars as well as pre-reproductive winged females).

Keywords: Spatial, temporal, stratified sampling, density dependence, *Trioxyspallidus*

Introduction

Iran is the most important walnut producer after China and followed by USA. Walnut production in Iran was around 485000 ton in 2011 (14.87% of the world production). Northwestern, western and northern regions of Iran are the most important Iranian walnut *Juglans regia* L. production areas (Anonymous, 2014). Among pests, two species of aphids, namely small walnut aphid (SWA) *Chromaphis juglandicola* (Kaltenbach), and large walnut aphid (LWA) *Panaphis juglandis* (Goeze) are well known, but little studied, ones that attack walnut trees. SWA is a holocyclic monoecious aphid that spends all of its life upon walnut trees. It spends winter conditions as hibernating eggs (Nowierski *et al.*, 1983). Exact amount of damage by this insect pest is unknown, but colonies under leaves in densities

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above 15/ leaf may cause leaf drop, fruits become sunburned and finally cause weakened trees and yield loss (Shelton and Davis, 1994; Mills *et al.*, 2011; Pickle *et al.*, 2015).

SWA has several natural enemies that can maintain populations well under economic levels (Pickle *et al.*, 2015). *Trioxys pallidus* (Haliday) (Hym. Braconidae, Aphidiinae) is a specific parasitoid of developed stages of SWA (Rakhshani *et al.*, 2002). *Dieretiella rapae* from the same subfamily is another generalist parasitoid that attacks SWA nymphs and adults (Strand, 2003; Shi and Chen, 2005). The former species is the most important specific parasitoid of this aphid (Mace and Mills, 2016). An Iranian population of this wasp was introduced into USA from hot central plateau of Iran in 1960 and was successful pest control agent in hot internal regions of California (Schlinger and Hall, 1961; van den Bosch *et al.*, 1979; Abivardi, 2001). Since 1969 successful control of walnut aphid has been achieved by this wasp throughout California (Messing and Aliniazee, 1989; Mills *et al.*, 2011). Many other generalist natural enemies feed on SWA. Seven species of ladybird beetles, green lacewing *Chrysoperla carnea* (Stephens) and a few species of predatory bugs are among predatory species whose predatory activity was recorded on SWA (Sluss, 1976; Ozkan and Türkyilmaz, 1987; Mehrvar et al., 2001; Strand, 2003; Margo *et al.*, 2010). These reports may reveal the importance of natural enemies in reducing SWA populations and the necessity of studying their relationships.

Population studies are important not only due to relationship between population density and damage and hence economic importance of a pest species (Dent, 2000), but also because these studies reveal relative importance of natural forces that control population levels (Manly, 1990; Price *et al.*, 2014). Furthermore these studies are necessary for timing of control measures during population release (Pedigo, 1999; Dent, 2000). In Iran few studies were conducted on population changes of SWA. For example Mehrvar *et al.* (2001) studied population fluctuations of SWA and its natural enemies at two locations around Tabriz, northwest Iran. They observed considerable variation in SWA population densities between the two years (1998 and 1999) at a single location. Also Jaskiewicz and Cichocka (2004), Jaskiewicz and Kmiec (2007), Karczmarz (2010; 2012) studied the relative importance of both species of *C. juglandicola*, and *P. juglandis* in Lublin, Poland. They reported that activity of both species was during May-October. Drought, temperatures above 30 ºC and rain showers were reported as the most important factors in summer population declines. Population peaks occurred in June or July in different years. Both temporal and spatial difference was observed in population. In two of three locations, *C. juglandicola* was dominant in 2003, while *P. juglandis* was dominant species in the remaining combinations of years and locations. In a laboratory study the population growth rate decreased above 30 ºC (Nowierski *et al.*, 1983). Talebi *et al.* (2002) studied on comparative population growth rate of SWA against its specific parasitoid, *T. pallidus*. In another study, population growth rate of SWA was reported to be much faster in summer cohort than that of spring cohort under outdoor condition. This was an unexpected result because natural populations often decline in summer. It was related to sap quality of young shoots used in that study in comparison to that of adult trees (Mahdavi *et al.*, 2012).

Previous studies by Nowierski and Gutierrez (1986a; b) on spatial distribution patterns of SWA eggs and adults in Tracy in 1977-78 revealed that egg density strongly varies with height and orientation of canopy, but no between-tree variation was detectible. No difference in aphid distribution was observed among the orientations, but a strong variation was observed along the branch. Most aphids aggregated on distal and median leaves rather than basal ones.

In this study, population fluctuations of SWA and its natural enemies were studied and correlation between them was investigated. Some factors like canopy orientation, branch length and height of canopy that were supposed to influence population levels were considered as three potentially effective factors.

Materials and Methods

Study site

This study was carried out in a walnut orchard (1 ha) located in Mamaghan, East Azarbaijan Province, Iran (ASL 37º 50' N, 45º 58' E). This region has been a main walnut grown region in the past two decades, since cultural pattern changed from almond to walnut.

Sampling design

Eight walnut trees were chosen randomly in a Z-shaped direction. A stratified sampling design was adopted with three sets of strata: 1) branch length at two levels (distal and basal ends), 2) vertical divisions of canopy at two levels (upper and lower half), and 3) the orientations of canopy at four levels (North, East, West and South displayed as N, E, W, and S respectively). Upper branches were accessed by a hooked stick. In this way the canopy of the chosen trees was divided into 16 strata (2 × 2 × 4) and a single sample was assigned to each stratum. Thus 128 units were inspected weekly. Samplings lasted from April to October 2012 (overall 30 times). Preliminary study showed that the *P. juglandis* was rare in the region; hence we focused on the more abundant species, *C. juglandicola*. All developmental stages of the aphid were counted *in sit*-in a cluster of leaves including five leaves of similar size, as a sampling unit. Developmental stages were detected visually by their size (Mahdavi et al., 2012). Sampling unit was reduced to a single leaf when population was released in late spring, because sampling measurements became very time-consuming. Moreover in order to save more time, data of the two sequential stages were pooled as first + second instars and third + fourth.

Determining overwintering stages

This study began in early November when 1/3 of walnut leaves had dropped in autumn while the aphid population reached zero. Previous studies had shown that eggs might be the overwintering stage (Nowierski *et al.*, 1983; Nowierski and Gutierrez, 1986a; b; Strand, 2003; Pickle *et al.*, 2015), but our observations did not wholly support these reports from other countries (no data was available for Iran). So all parts of bark as well as leaves (both fallen and live leaves) were eye-inspected using a handy lens to find the eggs. Simultaneously the other stages encountered were also recorded. Bark inspection was done by random look on different parts of trunk and branches for 2h in eight trees. Leaf inspection was done carefully on both sides of a leaf lamina as well as petiole. Counting the different stages of the aphid was done only among fallen leaves. Ten random sites in the orchard were defined and 100 leaves per each site were removed and checked for the overwintering individuals. This recording was weekly conducted for a month until the first snow. Overall samplings were done five times.

Surveying natural enemies

All flier predators were counted without handling branches prior to counting the aphid numbers. The ladybirds' eggs, larvae, pupae and adults were counted separately for the two species, *Coccinella septempunctata* L. and *Adalia bipunctata* (L.). All stages of the other predators including green lacewing *Chrysoperla carnea* Stephens, two species of spiders from the family Dictynidae, and *Orius* sp. (Hem. Anthocoridae) were counted as a single datum because of their fewness in number. Some specimens from each predator were removed and deposited in a vial (5ml) containing 2.5ml of 75% ethanol for further study and proper identification. Mummified aphids were removed and transferred to laboratory in similar empty vials for rearing parasitoids to obtain and identify the adults. Information such as location, number of tree, stratum and sampling date was labeled on vials.

Data analysis

A tree-way ANOVA as a 4 × 2 × 2 factorial design was adopted for determining possible tendencies of the walnut aphid to different strata. The post hoc test of Tukey's HSD test ($\alpha = 0.05$) was used for comparison of means. A separate analysis was done for each sampling date. The relevant SAS procedures (SAS Institute, 2011) were used in these analyses. The ratio of samples with significant tendency toward a stratum was determined per total sample.

In order to determine relationship between *T. pallidus* and aphid population fluctuations, a natural enemy per aphid ratio as well as a linear correlation between the two variables was investigated using Microsoft Excel. This analysis was done for developed stages of the aphid including third + fourth instar nymphs and adult winged females, because they were potential host stages which would be actually attacked by *T. pallidus* (Rakhshani *et al.*, 2004). In order to determine delayed effects of natural enemies, similar relationships were investigated between population levels of natural enemies, P_i in a determined week i, and those of the host 1, 2, 3, … weeks earlier $N_{i-\delta}$, where $\delta = 1, 2, 3, \ldots$ is time delay or lag in weeks (Manly, 1990). Logarithmic scale was used both for P and N:

$$\mathrm{Log}(P_i) = \alpha + \beta.\log(N_{i-\delta})$$

Results and Discussion

The SWA population fluctuations

Late April was the appearance onset of the SWA 1st instar nymphs on newly grown leaves (Table 1). Overall 18 nymphs (0.14 per leaf cluster) were counted in all samples on 27 April 2012. During subsequent weeks a gradual increase in number of 1st instar nymphs was observed, but appearance of light yellow winged females at the second week was amazing while no intermediate stage was present (Table 1). In all samples prior to 20 May 2012 no other stage was observed (Table 1). A possible explanation for priority of 1st instar nymphs to females is that number of females has been so few in the first sample that their detection in a sample as large as 128 cluster of leaves was impossible. Thus the 1st instar nymphs observed in the first week most probably have been progeny of those females that observed just one week later when their density reached to a level detectible by a sample of n = 128.The alternative hypothesis may be eclosion of overwintering eggs to born 1st instar nymphs and thus the SWA has had two overwintering forms in the region; eggs and developed stages. During the first month only light yellow females and lower stages of SWA were detectible and no co-incidence of all stages was observed (Table 1). This may show that the first generation took a month to complete its cycle. Females of all subsequent generations were light green. Overlapping of the two generations was observed at the end of May while all stages simultaneously were observed (Table 1). A sudden population release started at mid-May and continued up to late June. Incidence of population peak was on 2 June 2012 with 201 aphids/ sampling unit or 40/ leaf while 1st + 2nd instars were dominant. In subsequent weeks the more developed stages (winged females as well as 3rd + 4th instar nymphs) continued to increase while early stages began to decrease. Sudden decline occurred in late June. On 27 June 2012 population declined to 11% of the previous week. The possible reasons for such a decline might be weather warming (Nowierski *et al.*, 1983) or disqualified plant sap (Dixon, 1970; 1985) which is known as the most important driving force of aphids' fluctuations. Reproductive-life table studies by Mahdavi *et al.* (2012) also revealed effect of temperature and seasonal change on population growth of SWA. The other minor factor may be activity of natural enemies (see subsequent section). The population continued to decrease for further 2.5 months and reached to a minimum of 0.14 aphids/sampling unit on 7 September 2012, but never reached zero. A second increase began afterward, continued for a month and finally reached a minor peak of 2.13 aphids/sampling unit on 6 October 2012. Then population decreased slowly for a month and reached zero on 10 November. At this time 1/3 of walnut leaves had dropped. First instar nymphs disappeared two weeks earlier that may show cessation of natality in the autumn (Table 1). Time horizon and population peak coincided the Poland population of the SWA (Karczmarz, 2010; 2012). Also peak density was similar to earlier works (Mehrvar *et al.*, 2001).

Tendency toward directions of the canopy

In 40% of samples (12 out of 30) no tendency was detectible to any direction of canopy. This is both due to absence of seasonal tendency and small means (2/3 of non-significant samples approached zero); hence possible tendencies were undetectable. In the remaining 18 samples tendency was detectible only in some stages. This means 26 out of 117 *i. e.* 22% of stage-dates (Table 2). In more than half of the significant samples (15 out of 26), the aphids crowded at the northern part of canopy. However, this tendency was not absolute. This was true for those samples with higher means *i. e.* early stages at late May and early June. The higher density in these samples enhances the reliability of the estimates compared to the other samples. Moreover in most cases aphids abandoned the southern and western leaves (in 14 and 15 cases respectively; Table 2). Nowierski and Gutierrez (1986a) observed no tendency in different directions of canopy. This difference in their results may be due to differences in population means and life histories they experienced, difference in climates and other factors. A mysterious point in our study was a constant tendency of all immature stages to crowd the west side of canopy in sample of the 27th June. It may be a seasonal bias driven by asymmetrical movements of aphids. In other words aphids of different directions left the canopy at different times, hence, western canopy was depleted later.

Tendency along the branch

In 2/3 of the sampling dates a tendency was observed at least in some stages of the SWA along the branch (Table 3). This means 41 out of 117 combination of stage-dates or 35%. In all samples except one a constant tendency was observed toward basal end of branches. This is in contrast to Nowierski and Gutierrez (1986a) results in California. This may be due to more severe temperature fluctuations in our drier region, while driven force of population change in California is the sap quality. In other words aphids avoid hot temperatures and settled on more shaded strata in our region while they tended to younger leaves with better sap quality in California. The 1st instar nymphs revealed the highest tendency among the stages; in half of the samples this tendency was detectible. The higher number of the 1st instar nymphs made the differences became immediately evident.

Table 1 Population density of *Chromaphis juglandicola* (SWA) per five leaves in walnut orchards of northwest Iran, 2012.

Stages	Number of insects / 5 leaves														
	27-Apr	04-May	11-May	18-May	24-May	29-May	02-Jun	09-Jun	15-Jun	22-Jun	27-Jun	05-Jul	13-Jul	21-Jul	27-Jul
N1[1]	0.14	0.16	0.66	7.05	20.13	50.66[2]	132.59[2]	61.57[2]	90.57[2]	92.89[2]	13.46[2]	3.19	1.91	0.79	0.27
N2[1]	0	0	0	0.84	8.68							0.99	0.43	0.29	0.12
N3[1]	0	0	0	0	0.95	7.91[2]	62.47[2]	70.56[2]	59.09[2]	45.86[2]	1.55[2]	0.59	0.13	0.16	0.07
N4[1]	0	0	0	0	0							0.34	0.12	0.07	0.03
Adult	0	0.08	0.05	1.02	1.01	1.56	6.13	14.34	9.21	9.41	1.33	0.34	0.09	0.03	0.04

Table 1 Continues

Stages	Number of insects / 5 leaves														
	03-Aug	10-Aug	16-Aug	24-Aug	31-Aug	07-Sep	14-Sep	21-Sep	29-Sep	06-Oct	12-Oct	19-Oct	26-Oct	04-Nov	11-Nov
N1[1]	0.26	0.13	0.11	0.09	0.08	0.05	0.1	0.2	0.2	0.72	0.7	0.14	0	0	0
N2[1]	0.09	0.06	0.05	0.03	0.03	0.06	0.03	0.08	0.16	0.65	0.37	0.3	0.11	0.01	0
N3[1]	0.05	0.05	0.05	0.03	0.01	0	0.02	0.09	0.13	0.44	0.31	0.28	0.23	0.02	0
N4[1]	0.05	0.05	0.02	0.03	0.02	0.01	0.03	0.04	0.05	0.16	0.16	0.1	0.13	0.02	0
Adult	0.08	0.05	0.05	0.01	0.02	0.02	0.03	0.08	0.08	0.16	0.14	0.14	0.11	0.01	0

1. N1 to N4 displays nymphal stages of SWA, 2. pooled together for samples of 29 May to 27 June.

Table 2 Significant tendency of *Chromaphis juglandicola* (SWA) for different geographical directions of canopy in walnut trees of northwest Iran.

Stages	Tendency of insect to geographical directions																
	04-May	11-May	24-May	29-May	09-Jun	27-Jun	05-Jul	13-Jul	21-Jul	10-Aug	16-Aug	24-Aug	07-Sep	14-Sep	29-Sep	06-Oct	12-Oct
N1[1]	-	-	N>W, E, S	N>S +W	N>W	W>E, S	-	-	N>S	N>E	-	-	-	-	N>E, W	S>W	-
N2[1]	-	-	-	N>S +W	N>W	W>E, S	-	-	-	-	-	-	-	E>N, W	-	-	-
N3[1]	-	-	-	-	-	W>E, S, N	N>E, S	-		-	E>W, S, N	S>N, E	-	-	N>E, S, W	-	-
N4[1]	-	-	-	-	-	W>E, S, N	-	E, W>N	-	W>N	-	-	-	-	-	-	S>W
Adult	N>S	N>W	-	-	N>W, S, E	-	-	-	-	-	-	-	N>W, S, E	N>E, S	-	-	-

1. N1 to N4 displays nymphal stages of SWA.
2. N, E, W, S displays the four geographical orientations North, East, West and South respectively.

Table 3 Significant tendency of *Chromaphis juglandicola* (SWA) toward basal (B) or distal (D) end of branch in walnut trees of northwest Iran.

Stages	Tendency of insect to basal (B) or distal (D) end of branch																			
	11-May	18-May	24-May	29-May	02-Jun	15-Jun	22-Jun	27-Jun	05-Jul	21-Jul	03-Aug	10-Aug	16-Aug	24-Aug	07-Sep	14-Sep	21-Sep	29-Sep	06-Oct	12-Oct
N1[1]	-	-	B	B	-	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B
N2[1]	-	-	B	B	-	-	-	B	B	-	B	-	-	B	B	B	-	-	B	B
N3[1]	-	-	B	-	B	-	-	-	-	-	-	B	-	B	-	-	-	-	-	B
N4[1]	-	-	-	-	B	-	-		-	-	-	B	-	B	-	-	-	-	-	-
Adult	D	B	B	B	-	B	B	B	B	-	-	-	-	-	-		-	-	-	-

1. N1 to N4 displays nymphal stages of SWA.

Tendency along height of canopy

At least one stage in half of the samples distributed unequally between strata of lower and higher canopy (Table 4). The sample of 29 May 2012 is outstanding in presenting a constant tendency in all stages toward lower canopy. Totally, except for two sampling dates, the aphids were more crowded in lower parts of canopy than the upper parts. What is more, this tendency was not absolute because only in 24% (28 out of 117) of stage-dates such a tendency was detectible.

As a whole it seems that SWA partially prefers more shaded areas such as lower half, basal end and northern parts of canopy most probably because of a more favorable microclimate of these strata. Such a tendency also has been reported by some researchers in different insects including aphids (*e. g.* Wagner, 2001; Dixon and Hopkins, 2010).

Natural enemies

Richness

The parasitic wasp *T. pallidus* was dominant species of the natural enemies in the region. Overall 2408 specimens of natural enemies were collected during the season within which 2160 specimens *i. e.* 90% belonged to mummies of *T. pallidus*. The other natural enemies were seven-spotted ladybird *Coccinella septempunctata* L., two-spotted ladybird *Adalia bipunctata*, green lacewing *Chrysoperla carnea* Stephens, predatory bug *Orius* sp. and two species of spiders from Dictynidae that their interaction with the aphid is not known. This list of natural enemies is a small part of fauna related to SWA in literature (for example Sluss, 1976; Ozkan and Türkyilmaz, 1987; Mehrvar *et al.*, 2001; Strand, 2003; Rakhshani *et al.* 2002; Margo *et al.*, 2010). Among the predators, the two species of ladybirds made up 7% of the specimens with obvious superiority of *C. septempunctata* in number.

The natural enemy/ aphid population ratio was about 0.02, but it increased to 0.067 when only those aphid stages were considered that may potentially be attacked by natural enemies (3rd and 4th instar nymphs as well as winged females). If a single predator only consumes 9 aphids in its life span the impact of the predator will be equal to *T. pallidus*, but higher life time consumption is expected (Xue *et al*., 2009). It means a predator is expected to kill more than nine SWA, although some of them may leave the location before any handling. Of course daily consumption rate as well as instantaneous population growth rate of the two organisms also must be taken into account for an accurate comparison between their efficiency (Mills, 1997). In this comparison also the prey specificity and frequency of switching both among habitats and diets by predator also must be considered (van Alphen and Jervis, 1996; van den Assem, 1996). Because these bits of information is not available an intact prey comparison is not possible but it seems that the relative importance of predators must be higher than specific parasitoid *T. pallidus* (Sluss, 1976; Mehrvar *et al*., 2001).

Table 4 Significant tendency of *Chromaphis juglandicola* (SWA) toward upper (U) or lower (L) parts of canopy in walnut trees of northwest Iran.

Stages	Tendency of insect to upper (U) or lower (U) parts of canopy														
	24-May	29-May	09-Jun	15-Jun	22-Jun	27-Jun	05-Jul	13-Jul	21-Jul	16-Aug	07-Sep	14-Sep	29-Sep	06-Oct	19-Oct
N1[1]	L	L	-	L	L	L	L	-	L	L	L	L	L	-	-
N2[1]	-	L	-	L	L	L	-	-	-	-	-	-	-	L	-
N3[1]	-	L	U	-	-	-	-	U	-	-	-	-	-	L	L
N4[1]	-	L	U	-	-	-	-	-	-	-	-	-	-	-	-
Adult	-	L	U	L	-	L	L	-	-	-	-	-	-	-	-

1. N1 to N4 displays nymphal stages of SWA.

Seasonal fluctuations

Natural enemies were first recorded on 2 June with a density of 50/ 128 samples. It means over a month later than the appearance of host itself (Table 5). Initial number of natural enemies was high and coincided with host population release. This delay may partly be explained by absence of more developed stages of 3rd and 4th instar nymphs, which are actual stages attacked by natural enemies, in samples prior to 24 May. Yet natural enemies were delayed two weeks more. Following the emergence of natural enemies, increasing trend of SWA population ceased. Nevertheless no decrease in number of SWA was observed while natural enemies continued to increase for 3 weeks. The peak number of natural enemies was recorded on 22 June (685/ 128 samples). This represents a 3-week delay compared to the host peak. A sudden decline in host density as well as a moderate decrease in natural enemies' populations occurred afterward. Two sharp declines also occurred in natural enemies' population, first one on 13 July (3 week after severe decline of SWA populations), and the second time two weeks later. In each of these phases, natural enemies' population levels reached lower than half as much as the previous levels. Gradual decrease continued up to late September when SWA population began its autumnal increase. Interestingly minimum number of natural enemies was achieved again two weeks after SWA minimums and the second peak occurred on19 October (38/ 128 samples) two weeks later than SWA's autumnal peak. Natural enemy counts reached zero, a week sooner than SWA.

Density dependence and delayed response

As it was seen in previous section, majority of population events (emergence, seasonal peaks, minima, *etc.*) occurred 2-3 weeks later in natural enemies in comparison to SWA (Fig. 1). So we examined the hypothesis of delayed density dependence of *T. pallidus* (the dominant species of natural enemies). Such a response is common among natural enemies (Hassell, 1978; Donaldson *et al.*, 2007; Chacon and Heimpel, 2010, Price *et al.*, 2014). Direct density dependence was observed between SWA and *T. pallidus* populations (R^2 = 0.389, P = 0.0019). Also significant correlations were observed between populations of the two insects by taking 1-8 weeks lag into account (Table 6). Maximum correlation was obtained when 2-4-week lag was taken into account. Difference between these lags was negligible and this may imply a 2-4 week delay in *T. pallidus* response. This time may be considered as the time required for development of mummies to emerge. An anticlockwise curve delineating relationship between log numbers of SWA and log number of *T. pallidus* mummies (or the same SWA mortalities, Fig. 2) may confirm delayed density dependent response of *T. pallidus* (Manly, 1990; Southwood and Henderson, 2000).

Table 5 Counts of different stages of *Chromaphis juglandicola* and its natural enemies in 128 samples taken from walnut orchards of northwest Iran, at different dates.

Entries	Number of insects / 128 leaves										
	02-Jun	09-Jun	15-Jun	22-Jun	27-Jun	05-Jul	13-Jul	21-Jul	27-Jul	03-Aug	10-Aug
Total population	25751	18749	20336	18965	2091	681	342	171	68	68	43
3rd and 4th instar nymphs	7996	9032	7564	5870	198	103	31	29	13	13	13
Adults	784	1836	1169	1205	170	43	11	4	5	10	6
NE's[1]	50	84	307	685	490	314	145	114	40	34	13
T. pallidus	38	67	281	590	442	288	144	113	39	32	13

Table 5 Continued

Entries	Number of insects / 128 leaves										
	16-Aug	24-Aug	31-Aug	07-Sep	14-Sep	21-Sep	29-Sep	06-Oct	12-Oct	19-Oct	26-Oct
Total population	36	25	21	18	27	62	79	271	215	124	74
3rd and 4th instar nymphs	9	8	4	1	6	17	23	76	60	49	46
Adults	6	1	3	3	4	10	10	20	18	18	14
NE's[1]	16	9	11	12	6	8	4	10	7	38	11
T. pallidus	13	9	8	10	5	7	4	10	5	32	10

1. NE presents all natural enemies (parasitoid *T. pallidus* + predators combined). NE's minus *T. pallidus* may show predators. For example in the first sample 50 NE's minus 38 parasitoid gives 12 predators.

Table 6 Correlation between *Chromaphis juglandicola* population (3^{rd} + 4^{th} instar nymphs + females) and mortality caused by *Trioxys pallidus*, taking 0-9 weeks lag into account.

Parameters	0	1	2	3	4	5	6	7	8	9
r^2	0.389	0.647	0.874	0.896	0.902	0.789	0.624	0.461	0.296	0.16
F	12.76	34.82	124.99	146.43	147.38	56.25	23.27	11.13	5.03	2.02
P	0.002	1.11×10^{-5}	1.56×10^{-9}	8.85×10^{-10}	1.74×10^{-9}	1.89×10^{-6}	0.0003	0.005	0.045	0.18

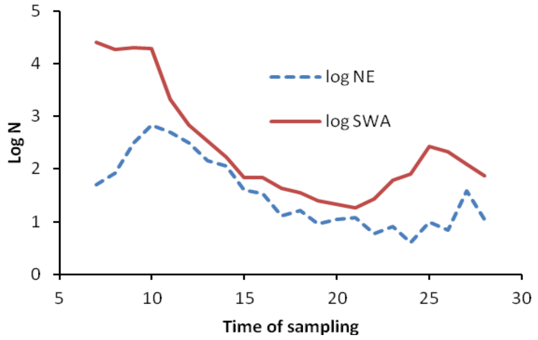

Figure 1 Population fluctuations of all stages of *Chromaphis juglandicola* (SW) and *Trioxys pallidus* (NE) combined at northwest Iran, during season 2012.

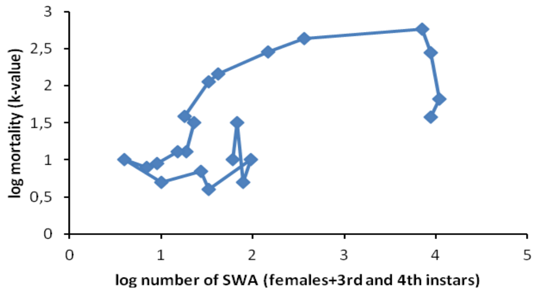

Figure 2 Anticlockwise curve of mortality caused by *Trioxys pallidus* against population density of *Chromaphis juglandicola* (SWA).

Overwintering stages of SWA

No more than two overwintering eggs (orange in color) were found among the samples. Majority of overwintering stages were 3rd and 4th instar nymphs followed by a lower number of 2nd instars and winged females (Table 7). These records confirmed our guess about the more developed stages of hibernating forms of SWA in the region and may demonstrate an obvious contrast to previous reports. Previous works (Nowierski *et al.*, 1983; Nowierski and Gutierrez, 1986a; b; Strand, 2003; Pickle *et al.*, 2015) had shown that egg is hibernating stage of SWA. Presence of winged females among early stage nymphs does not permit us to accept that these females were born from overwintering eggs. Such a difference may be attributed to a different geographical population with different life history. This may emphasize that information on life history events of an insect in some parts of the world may not necessarily correspond with those for other regions.

Table 7 Population density of overwintering stages of *Chromaphis juglandicola* recorded on fallen leaves of walnut trees during autumn 2012 at northwestern Iran.

Stages	Number of insects / leaf				
	11-Nov	18-Nov	25-Nov	02-Dec	11-Dec
Adult	4	4	1	-	-
4th instar	8	10	6	9	7
3rd instar	8	9	8	6	3
2nd instar	3	5	5	1	-

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تغییرات جمعیت شته‌ی کوچک گردو *Chromaphis juglandicola* (Hemiptera: Aphididae) و دشمنان طبیعی آن در باغات گردوی شمال غرب ایران

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چکیده: شته‌ی کوچک گردو *Chromaphis juglandicola* آفت اقتصادی درختان گردو می‌باشد. نوسانات جمعیت این آفت در باغات گردوی ممقان (استان آذربایجان شرقی) بررسی شد. اثر سه عامل شامل ارتفاع تاج درخت (بالا و پایین)، جهات چهارگانه درخت و بن و انتهای شاخه بر تغییرات جمعیت این شته مطالعه گردید. واحد نمونه‌گیری یک دسته پنج‌تایی برگ بود. در مجموع ۱۲۸ نمونه از تمام لایه‌ها از ۸ درخت گردو هر هفته تا آخر فصل گرفته شد و این کار به‌مدت ۳۰ هفته تکرار گردید. دشمنان طبیعی نیز شمارش گردیدند. نخستین افراد شته متشکل از پوره‌های سن اول و ماده‌های بالدار در اواسط اردیبهشت ملاحظه شدند. اواخر اردیبهشت افزایش ناگهانی جمعیت مشاهده شد. حداکثر ۴۰ شته در هر برگ در اواسط خرداد شمارش گردید که پوره‌های سن اول و دوم غالب بودند. در اوایل تیر نزول ناگهانی جمعیت اتفاق افتاد. هم‌چنین اواسط مهر یک افزایش جمعیت مختصر رخ داد. یک گرایش نسبی به‌سمت برگ‌های قاعده‌ای شاخه‌های زیری در جهت شمالی تاج درخت ملاحظه شد. به‌نظر می‌رسد میکروکلیمای مرطوب و سایه‌دار این لایه‌ها علت این گرایش بوده باشد. هم‌بستگی خطی مثبت بین جمعیت دشمنان طبیعی و شته دیده شد که نشان دهنده‌ی وابستگی به تراکم آن‌ها است. مضافاً ۴-۲ هفته تأخیر در تغییرات جمعیت میزبان و دشمنان طبیعی مشاهده گردید. گونه‌ی غالب دشمن طبیعی *Trioxys pallidus* (Holliday) بود. برخلاف مطالعات قبلی، مراحل زمستان‌گذران این شته پوره‌های سنین بالا و ماده‌های بالدار در مرحله‌ی پیش از تولیدمثلی تعیین گردید.

واژگان کلیدی: وابستگی به انبوهی، زمانی، مکانی، نمونه‌برداری لایه‌بندی شده، *Trioxyspallidus*