

Research Article

Population fluctuations of the Walnut aphids *Chromaphis juglandicola* and *Panaphis juglandis* (Hemiptera: Aphididae) and their natural enemies in Farokhshahr and Saman walnut orchards, Southwestern Iran

Arsalan Khalili-Moghadam^{1, 2*} and Zarir Saeidi³

- 1. Plant Protection Department, Agriculture College, Shahrekord University, Shahrekord, Iran.
- 2. Institute of Biotechnology, Shahrekord University, Shahrekord, Iran.
- 3. Plant Protection Department, Agricultural and Natural Reaserch Center, Shahrekord, Chaharmahal va Bakhtiari Province, Iran.

Abstract: Walnut aphids, the small walnut aphid Chromaphis juglandicola (Kaltenbach), and the large walnut aphid Panaphis juglandis (Goeze) are economic pests of walnut trees in Chaharmahal va Bakhtiari province Southwestern Iran. The population fluctuations of walnut aphids and their natural enemies were studied under field conditions over two successive years, 2022 and 2023. The sampling unit was a cluster of five leaves. A total of 100 samples were taken weekly from all strata upon ten chosen trees. In both years, the small walnut aphid population was continuously increasing and reached its maximum density during mid-May to late June, while the population of large walnut aphid had a short peak between mid-May and mid-June or mid-July. Field studies showed that both walnut aphids were overwintered as eggs in small seams and cracks of walnut, especially at the bases of fallen leaves. Overwintering eggs were hatched depending on the climate conditions during the third decade of March in Saman orchard and the first decade of April in Farokhshahr orchard. Our findings may provide practical information about the seasonal abundance of walnut aphids, which is useful for agricultural experts and farmers to develop effective integrated pest management (IPM) programs.

Keywords: walnut, aphid, seasonal activity, climate condition, Iran

Introduction

Walnuts are one of the world's most valuable garden products. Iran, with approximately 174,000 hectares and 292,564 tons in 2022, is the most critical walnut producer after China and the US (DPEIC, 2022; FAO, 2022). This dried fruit is one of the most important horticultural crops in Chaharmahal va Bakhtiari province (7954 tons in

2022), which is cultivated on an area of more than 8,000 hectares (DPEIC, 2022). Walnuts are infested by various pests, including mites, leopard moths, and aphids (Iranipour *et al.*, 2018; Saeidi and Nemati, 2020; Saeidi *et al.*, 2022; Khalili-Moghadam and Saeidi, 2023). Among pests, two species of aphids, the small walnut aphid *Chromaphis juglandicola* (Kaltenbach) and the large walnut aphid *Panaphis juglandis* (Goeze),

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cause a serious threat to walnut, particularly during the spring, and reduce the quality and quantity of the nuts (Shelton and Davis, 1994; Mills et al., 2011; Pickle et al., 2015). P. juglandis is much larger than C. juglandicola and lives entirely on the upper surface of leaves, while C. juglandicola feeds on the lower surface of the leaves. Both aphids obtain their nutrition from the phloem tissue and are found in Europe, Central Asia, India, Pakistan, China, and North America (Wani and Ahmad, 2014a).

The adult and immature stages of the walnut aphids damage foliage and fruit by sucking plant sap from the leaves and swollen buds. These pests produce honeydew that covers leaves and causes leaf drop, sunburned fruits, loss of productivity of staminate flowers (catkins), reduced walnut size, and increased shriveling of kernels, perforation of shells, and adhering hulls. Finally, this causes weakened trees and yield loss, and the quantity and quality of the fruits are significantly reduced (Barnes and Moffitt, 1978; Van den Bosch *et al.*, 1979; Shelton and Davis, 1994; Mills *et al.*, 2011; Wani and Ahmad, 2014b; Pickle *et al.*, 2015).

The economic threshold for the walnut aphid is considered to be 15 aphids per leaflet, and higher densities early in the season reduce nut yield and quality and cause an increase in nuts with perforated shells (Sibbett et al., 1982; Pickle et al., 2015), while infestation in the summer decreases nut quality (Pickle et al., 2015). If 10% to 15% of the leaflets are infested for 3 to 4 weeks before shell hardening, the nut size is decreased. It has also been reported that the same level of infestation during late summer results in shriveled kernels at harvest time and that treatment should be considered whenever an average of 10% of the subterminal leaflets have large walnut aphid colonies of six or more feeding on their upper surface (Pickle et al., 2015).

Ecologically, pest management programs require a greater understanding of the effect of climatic and weather changes on pests and interactions between pests and their natural enemies. Khaliq *et al.* (2014) stated that climatic and weather changes not only affect the status of insect pests but also impact their population dynamics, distribution, abundance, intensity, and

feeding behavior. Therefore, knowledge about the seasonal population dynamics of pests and their natural enemies is crucial for preparing effective control programs. Temperature, humidity, the quality of plant sap, and natural enemies are the most significant factors that influence the distribution and seasonal activity of walnut aphids under field conditions. It seems low temperatures and high humidity are suitable for walnut aphid's development and outbreak in agro-ecosystem (Sluss, 1967; Dixon, 1970, 1985; Nowierski et al., 1983; Mehrvar et al., 2001, Mahdavi et al., 2012; Atlihan et al., 2015; Iranipour et al., 2018). Moreover, the use of synthetic insecticides may lead to the development of insecticide resistance in the walnut aphid population and disrupt their natural enemies, especially Trioxys pallidus (Holliday), thereby increasing the walnut aphid population and damage (Mehrvar et al., 2001).

Atlihan et al. (2015) stated that P. juglandis causes damage to walnuts, especially between early and mid-summer. After that, its density decreases. They stated that parasitoids, predators, higher maximum temperatures in mid-summer, and the application of pesticides to control the codling moth may be important factors in the decrease in aphid densities (Atlihan et al., 2015). The population of the small walnut aphid is greatly affected by changing weather parameters, particularly in early spring with warm weather (less than 30 °C) and low precipitation, which enhances growth (Nowierski et al., 1983; Karczmarz, 2010, 2012; Gull et al., 2023). Gull et al. (2023) stated that humidity played a negative role, while little rainfall showed a positive correlation in population dynamics. Rakhshani et al. (2000) stated that population indices of C. juglandicola during the growing season showed a spring peak, followed by a rapid decline in summer and an ultimate increase in fall. In another study, the population of C. juglandicola increased suddenly in mid-May, followed by a rapid decrease in late June. A small peak was observed in early October (Iranipour et al., 2018).

Due to the walnut aphids causing serious damage to agricultural ecosystems, the present investigation was carried out to study the seasonal occurrence and population dynamics of walnut aphids under field conditions in Chaharmahal va Bakhtiari Province, Iran. Results can be useful in understanding the seasonal activities of walnut aphids and facilitating the development of a successful integrated pest management (IPM) program to control these pests under field conditions.

Materials and Methods

Location of study

Population fluctuations of walnut aphids were studied during two successive years, 2022-2023. The field experiments were conducted in Saman (32° 30' 44" N, 50° 12' 01" E, 1888 m a.s.l.) and Farokhshahr (32° 15' 08" N, 50° 58' 19" E, 2118 m a.s.l.) orchards, Chaharmahal va Bakhtiari province, Iran. Saman and Farokhshahr are the northeastern and eastern towns of Chaharmahal va Bakhtiari province, located in southwestern Iran, in the central part of the Zagros Mountains.

The orchards are located 3.5 and 6.5 km from synoptic stations in Saman and Farokhshahr, respectively. Annual mean temperature and precipitation of synoptic stations are 13.35, 12.5 °C, and 279.4, 272 mm in synoptic stations of Saman and Farokhshahr, respectively.

The weather in the studied areas is cold in winter, cool in spring and fall, and temperate in summer. Rainfall season generally starts in the second decade of November and ends in the last decade of May. January and February are the coldest months, whereas June and July are the hottest months in the region.

Field studies

Population sampling

Two commercial orchards of walnut, with a distance of 50 km, were selected (one orchard in Saman and another in Farokhshahr), and no insecticides were sprayed during the period of study. The orchards were almost uniform in terms of walnut trees, soil texture, and nutrition management.

The observations on the incidence and fluctuation of walnut aphids, *C. juglandicola* and *P. juglandis*, were recorded every week (although the graphs were drawn using data from

every two weeks). This period began in April and continued through October.

For the first sampling, 20 trees in each orchard were selected randomly, and 15 random leaves from different portions (at different sides) of each tree were collected. Different life stages of the walnut aphids (nymph and adult) and their natural enemies were counted on both leaf surfaces of the walnut. To calculate the sample sizes of second units (leaves) in subsequent sampling intervals, the random sampling method was used, considering 20 trees in each sampling (Krebs, 1989).

$$N = \left(\frac{Z\alpha_{/2}}{D}\right)^2 \left(\frac{\sigma^2}{\mu^2}\right)$$

Where,

N: sample size.

D: constant error (D = 0.2).

 σ^2 and μ is the population variance and mean from the previous sampling, respectively.

Overwintering study

To determine the overwintering stage and hatching time of the overwintering eggs, sampling was done weekly from October to April. For this purpose, 10 trees in each orchard (site) were selected randomly, and five branches (20 cm in length) from different sides of each tree were collected and transferred to the laboratory. The collected branches were placed in plastic containers and incubated at 25 ± 1 °C and $50 \pm 5\%$ RH. To keep the sample fresh, a layer of cotton wad soaked in water is placed at the bottom of the container. Samples were observed daily under a binocular microscope at 10X magnification, and the egg status was recorded.

Meteorology data

Daily temperature (mean, maximum, and minimum) and rainfall data for Saman and Farokhshahr synoptic stations were collected from the Chaharmahal va Bakhtiari Meteorological Office.

Statistical analysis

The data obtained from the seasonal activity of two walnut aphids, the relationship between aphid population and parasitoid densities, and environmental temperature were analyzed using Excel software.

Results

Field studies

Population fluctuations of small walnut aphid *C. juglandicola*

Overwintering eggs were hatched in the first decade of April in Farokhshahr and the last decade of March in Saman in both years (2022 and 2023). The mean temperatures of the mentioned times were measured at 10.6 °C and 9.6 °C in Saman and 14 °C and 9.6 °C in Farokhshahr orchards in 2022 and 2023, respectively. In 2022, no rainfall occurred at this time. In contrast, 2.8 and 0 mm of rainfall were recorded in Farokhshahr and Saman sites, respectively, in 2023.

In 2022, at the Saman site, the aphid population began increasing from the third week of May and reached its first maximum density (22.5 aphids per leaf cluster) in the last week of May. It then suddenly declined to 15.78 aphids per leaf cluster by 10th June. The population increased and reached its second maximum density (24.76 aphid/leaf cluster) on June 21st and then rapidly declined to its minimum value (0.06 aphid/leaf cluster) from the last decade of July to the first decade of September. Although the population increased slowly in mid-September, it decreased again at the beginning of the cold season (late October) and started laying over-wintering eggs (Fig. 1).

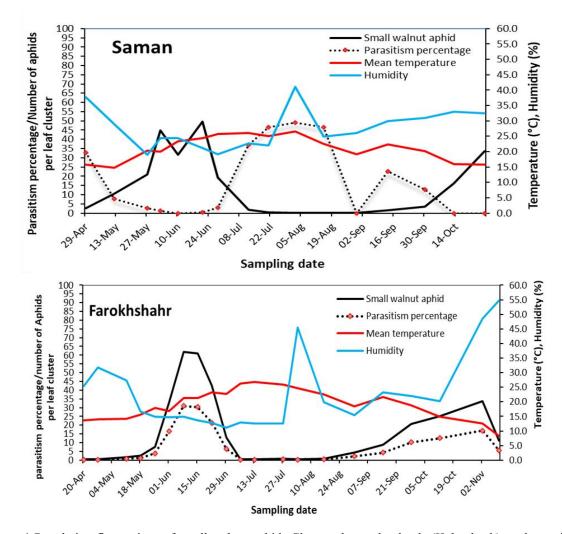


Figure 1 Population fluctuations of small walnut aphid, *Chromaphis juglandicola* (Kaltenbach), and parasitism percentage (*Trioxys pallidus*) under the field conditions (divided by 2) in Saman and Farokhshahr regions, Chaharmahal va Bakhtiari province during 2022.

The aphid population at the Farokhshahr site continuously increased and reached its maximum density (61.95 aphid/leaf cluster) on June 8th, and then rapidly declined to its minimum value (0.5 aphid/leaf cluster) in the first decade of July to mid-August. After that, the aphid population continued to increase slightly, but in late October, it decreased again and started laying over-wintering eggs (Fig. 1).

In 2023, in the Saman site in mid-May, the aphid population continuously increased and reached the maximum density (23.82 aphid/leaf

cluster) in the fourth week of July and then rapidly declined to the minimum value (0.24 aphid/leaf cluster) in mid-August (Fig. 2). Totally, in Farokhshahr site's aphid population was more than Saman site's. Although in this site, the population was low from the beginning of the generation season to the first decade of August, suddenly, the population continuously increased and reached the maximum density (79.34 aphid/leaf cluster) on 04th September, and then slowly declined to the minimum value (1.78 aphid/leaf cluster) in 08th November (Fig. 2).

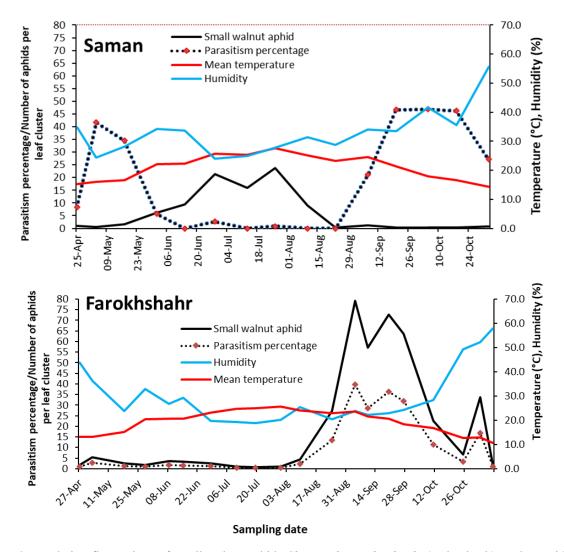


Figure 2 Population fluctuations of small walnut aphid, *Chromaphis juglandicola* (Kaltenbach), and parasitism percentage (*Trioxys pallidus*) under the field conditions (divided by 2) in Saman and Farokhshahr regions, Chaharmahal va Bakhtiari province during 2023.

Population fluctuations of large walnut aphid *P. juglandis*

The population of the large walnut aphid was lower than that of the small walnut aphid in both sites especially in the Farokhshahr orchard (in most sampling dates, it was close to zero). In the Farokhshahr site, a short peak was observed between mid-May to mid-June in 2022 (Fig. 3), whereas a peak from the last week of May to mid-July in 2023 (Fig. 3). Then the aphid population declined to a minimum value (close to zero aphid/leaf cluster) the last decade of October. After that, a small peak was observed

between the last week of October and the first week of November in both studied years (Fig. 3). In Saman, a large peak was observed between last week of May and mid-July in 2022 (Fig. 4), whereas, it was between mid-May to mid-July in 2023 (Fig. 4). Then the aphid population declined to minimum value (close to zero aphid/leaf cluster) in early September. After that, a minimal peak was observed between late September and early October in both studied years (Fig. 4). Hatching time of the overwintering eggs was similar to that of a small walnut aphid.

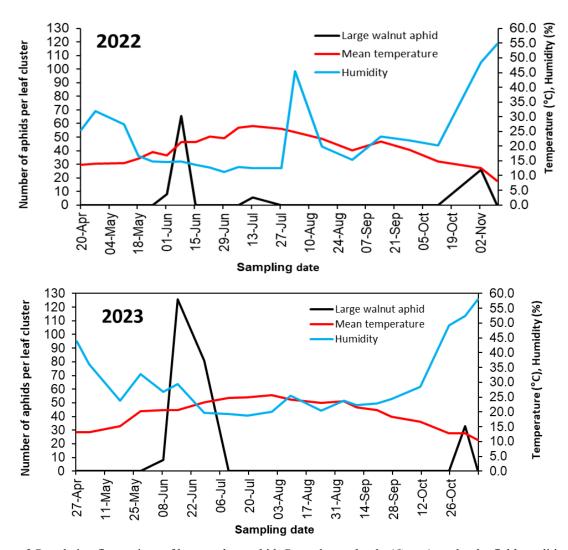


Figure 3 Population fluctuations of large walnut aphid, *Panaphis juglandis* (Goeze), under the field conditions in Farokhshahr region, Chaharmahal va Bakhtiari province during 2022 and 2023.

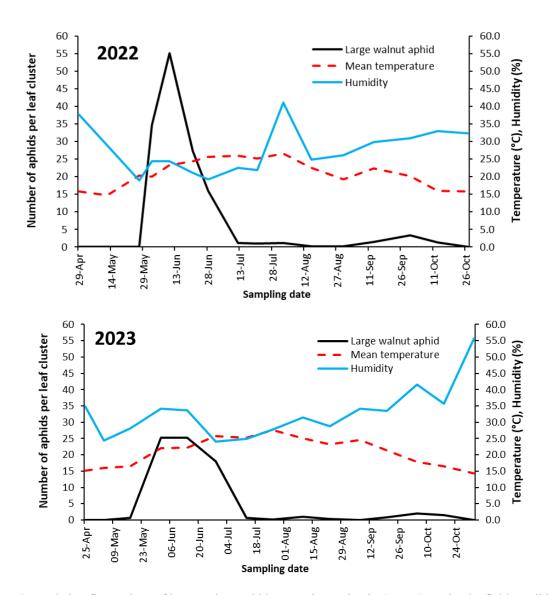


Figure 4 Population fluctuations of large walnut aphid, *Panaphis juglandis* (Goeze), under the field conditions in Saman region, Chaharmahal va Bakhtiari province during 2022 and 2023.

Parasitism percentage (*Trioxys pallidus*)

Parasitism percentage by *T. pallidus* on small walnut aphids was low on most days of green season in both sites and years (Figs. 1). However, the parasitism percentage reached to a maximum of 96 and 84 percent in Saman and Farokhshahr respectively, in 2022 (Figs. 1), whereas to 94 and 86 percentage in Saman and Farokhshahr respectively in 2023 (Figs. 2). Moreover, a coccinellid species, *Adalia* sp., (Coleoptera: Coccinellidae) a hoverflies species, *Syrphus* sp., (Diptera: Syrphidae) and

green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), and one species of spider, as predator were active in both sites. We didn't observe the parasitism behavior of *T. pallidus* on large walnut aphids in either site or year.

Our observations in this study revealed that environmental factors, particularly temperature, significantly influence the population fluctuations of walnut aphids (r = 0.73, p = 0.01). In early spring, due to rainfall and low temperatures, the pest population began to

increase slowly. In contrast, the gradual increase in temperature during April and early May led to a rapid increase in aphid populations from May to June, and then to early July. After that, due to the sudden increase in temperature, the aphid population rapidly decreased until September (2022) (Fig. 1). In 2023, the population increased more slowly in spring due to more rainfall days and low temperatures; therefore, the highest population density was observed in mid-August and then decreased.

Moreover, as the temperature dropped in October to less than 17 °C, aphid population growth declined, and females gradually moved to shoots that were 2 or more years old. They laid overwintering eggs in small seams and cracks (especially at the bases of fallen leaves of the last year) till the end of November.

As shown in Fig. 2, the spring rains affected the population density of the walnut aphid. More rainfall days in spring increased the humidity and decreased the temperature. Following these conditions, the aphid population increased, but the peak of the population was delayed.

Overwintering

Seasonal activities and population fluctuations of walnut aphids C. juglandicola and P. juglandis under natural conditions showed that these aphids overwintered in the form of eggs in small seams and cracks, especially at the bases of fallen leaves. Results showed that diapaused overwintering eggs did not hatch before March. Samples collected weekly from late November to 1st decade of March were not hatched when incubated at optimum conditions (25 ±1 °C, 50±5 % RH) in the laboratory. In contrast, those collected after second decade of March hatched 10 days later. Under natural conditions, overwintering eggs were hatched depending on the climate conditions during the first decade of April in Farokhshahr and the last decade of March in Saman in both studied years (2022 and 2023). The average winter temperatures for 2022 and 2023 were recorded in Saman at 1.47 °C and 5.13 °C, and in Farokhshahr at 1.07 °C and 4.47 °C, respectively.

Discussion

Walnut aphids, *C. juglandicola* and *P. juglandis*, have been significant pests of walnut trees in Chaharmahal and Bakhtiari provinces, Iran. In this study, *C. juglandicola* and *P. juglandis* were observed as the dominant species on walnut foliage at the Farokhshahr and Saman sites, respectively. Farokhshahr sites (located in the plain) supported a higher density of small walnut aphid population. In comparison, the Saman sites (situated along the Zayanderood River) had a higher density of a large walnut aphid population.

According to current investigations, cool weather and high relative humidity provide suitable conditions for increasing the aphid population. The aphid population rapidly declined to its minimum value by the end of August following an increase in temperature from late June to early September. It then slowly increased as the temperature decreased in early October, which is consistent with the results of Rakhshani et al. (2000), Karczmarz (2010, 2012), Atlihan et al. (2015), Iranipour et al. (2018), and Gull *et al.* (2023). However, due to varying environmental conditions (especially temperature), the periods of increasing and decreasing population density may differ in different regions.

Based on our observation, walnut aphids overwintered in the form of eggs in small seams and cracks, especially at the bases of fallen leaves, which they had diapause and did not hatch before March, similar to the results of Atlihan et al. (2015) in Turkey and Nowierski et al., (1983) and Pickle et al. (2015) in California and Wani and Ahmad, 2014b in India. Our result showed that overwintering eggs were hatched in the first decade of April in Farokhshahr and the last decade of March in Saman in both studied years, in contrast to Atlihan et al. (2015), who stated that egg hatching occurred from April to June in Turkey, which is probably due to the lower temperature in Turkey. According to Hougardy and Mills (2008), the lower thermal threshold of a small walnut aphid is 8.7 °C. Based on the meteorological data (Figs. 1-2), the mean temperature of the first decade of April in Farokhshahr and the last decade of March in Saman in both years of study increased above this level. The mean temperature of the last decade of March in Farokhshahr and the second decade of March in Saman was less than the lower thermal threshold of a small walnut aphid (8.7 °C).

In this study, the population density of natural enemies was very low, which may be due to the use of insecticides against other important pests in recent years. Trioxys pallidus was the most abundant and active natural enemy in our study, but it was not effective against the pest population. In contrast, the fauna of natural enemies in Turkey's orchards was strong, and they appeared to be effective in suppressing aphid populations to low levels (Atlihan et al., 2015). Population peaks of mummies of C. juglandicola occurred late in the season (between late summer and fall), at which time the density of aphids was low in both sites and years. Similar to our results, the highest parasitization rates by T. pallidus on C. juglandicola were recorded in the late season (Nowierski, 1979; Atlihan et al., 2015).

Nevertheless, some natural enemies, such as a coccinellid species (*Adalia* sp.), a hoverfly species (Syrphus sp.), the green lacewing *Chrysoperla carnea* (Stephens), and one species of spider, were active and fed on walnut aphids, whose efficiency should be studied.

This is the first study in southwest Iran to present population fluctuations of two walnut aphids on walnut trees under natural conditions, providing useful information for other researchers interested in various biological aspects of the walnut aphids. Moreover, it provides practical information about the seasonal abundance of walnut aphids and their associated natural enemies, which may be useful for agricultural experts and farmers to develop a successful integrated pest management (IPM) program.

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Authors' Contribution

All authors contributed to the conception and design of the study. Material preparation, data

collection, and analysis were performed by [Arsalan Khalili-Moghadam] and [Zarir Saeidi]. The first draft of the manuscript was written by [Arsalan Khalili-Moghadam], and all authors commented on it.

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Conflict of Interest

The authors declare no conflict of interest.

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تغییرات جمعیت شتههای گردو (Hemiptera: Aphididae) و Chromaphis juglandicola (Hemiptera: Aphididae) و دشمنان طبیعی آنها در باغهای گردوی فرخشهر و سامان، جنوب غربی ایران

ارسلان خلیلی مقدم ۲۰۱ و زریر سعیدی ۳

۱- گروه گیاه پزشکی، دانشکده کشاورزی، دانشگاه شهر کرد، شهر کرد، ایران.

۲- پژوهشکده بیوتکنولوژی، دانشگاه شهر کرد، شهر کرد، ایران.

۳- بخش تحقیقات گیاهپزشکی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی چهارمحال و بختیاری، سازمان تحقیقات، آموزش و ترویج کشاورزی، ایران.

يست الكترونيكي نويسنده مسئول مكاتبه: arsalan.khalili@gmail.com

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چکیده: شتههای گردو، شته کوچک گردو (Kaltenbach) و شته بزرگ گردو و در استان چهارمحال و بختیاری در جنوب عربی ایران هستند. تغییرات جمعیت شتههای گردو و دشمنان طبیعی آنها در دو سال متوالی ۲۰۲۲ و غربی ایران هستند. تغییرات جمعیت شتههای گردو و دشمنان طبیعی آنها در دو سال متوالی ۲۰۲۳ و غربی ایران هستند. تغییرات جمعیت شتههای گردو و دشمنان طبیعی آنها در دو سال متوالی ۲۰۲۳ مجموع ۱۰۰ نمونه در هفته از تمامی طبقات بر روی ده درخت انتخاب شده برداشت شد. در هر دو سال جمعیت شته کوچک گردو بهطور مداوم افزایش یافت و در اواسط اردیبهشت تا اواخر خرداد به حداکثر تراکم رسید و جمعیت شته بزرگ گردو بین اواسط اردیبهشت تا اواسط خرداد یا اواسط تیر اوج کوتاهی داشت. مطالعه مزرعهای نشان داد که هر دو شته گردو بهصورت تخم در درزهای کوچک و شکافهای گردو بهویژه در پایه برگهای افتاده زمستانگذرانی میکنند. تخمهای زمستانگذران بسته به شرایط اقلیمی در دهه سوم اسفند در باغ سامان و دهه اول فروردین در باغ فرخشهر تفریخ شدند. یافتههای ما ممکن است اطلاعات کاربردی در مورد فراوانی فصلی شتههای گردو ارائه دهد و برای کارشناسان کشاورزی و کشاورزان مفید باشد تا یک برنامه مدیریت تلفیقی آفات (IPM) را توسعه دهند.

واژگان کلیدی: گردو، شته، فعالیت فصلی، وضعیت آب و هوا، ایران