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Seasonal Activity and Damage Caused by Leopard Moth, *Zeuzera pyrina* L., in Walnut Orchards, Chaharmahal va Bakhtiari Province, Iran

Z. Saeidi[1*], A. Bagheri[2], and A Khalili-Moghadam[3]

ABSTRACT

Seasonal flight of the adults, larval activity, and damage caused by Leopard moth, *Zeuzera pyrina* L., was studied in the walnut orchards in Chaharmahal va Bakhtiari province, Iran. Pheromone baited traps were set up in the studied orchards from May 10 (before the emergence of adult males) to July 15 (the end of the adults' flight) to monitor the flight dynamics. Observation on development of different stages of the pest was done weekly throughout the growing season. The infestation ratio of twigs and number of active galleries per tree were recorded at studied locations. According to the results, the pest completed its life cycle within a year and overwintered as 4th and 5th instar larvae inside the galleries in trunk and main branches of walnut trees. The flight season of *Z. pyrina* started in the second decade of May, the peak occurred in the first decade of June, and the flight ended by the second decade of July. Eggs were laid individually next to the buds on the twigs and 1st instar larvae tunneled directly into inner wood. The maximum damage in shoots and twigs was observed at the third decade of August and, thereafter, larvae gradually moved to the main branches and trunk. Results showed that the biology and seasonal flight pattern of *Z. pyrina* in this province are different from other infested areas and that may be because of the climate condition. The knowledge of the observed differences may be useful to develop a successful integrated management strategy against *Z. pyrina*.

Keywords: Life cycle, Seasonal flight dynamic, Geographical difference, Woodborer insect.

INTRODUCTION

The leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae), is a dangerous wood borer insect and is considered as the most important pest of the walnut in Iran and other countries (Esmaili, 1991; Radjabi, 2002; Kutinkova *et al.*, 2006). It is also reported as the serious pest of olive in the North Africa (Katsoyannos, 1992) and Egypt (Hegazi *et al.*, 2009), apple in Syria (Almanoufi, 2012), Iran (Esmaili, 1991; Radjabi, 2002), Bulgaria (Kutinkova *et al.*, 2006, 2009), Spain (Sarto-Monteys, 2001) and Italy (Pasqualini and Natale,1999) and hazel nuts in North East of Spain (Isart *et al.*, 1997). A wide variety of trees and shrub from over 150 plant species and 20 genera such as pear, apple, walnut, cherries, hazel nuts, maple, willow, musk willow, elm and ash are attacked by the pest (Carter, 1984; Gatwick, 1992; Kutinkova *et al.*, 2006).

The larvae of *Z. pyrina* cause serious damage to the trees by boring into the twigs,

[1] Plant Protection Department, Agricultural and Natural Resources Research and Education Center, Chaharmahal va Bakhtiari, AREEO, Shahrekord, Islamic Republic of Iran.
*Corresponding author, e-mail: z.saeidi@areo.ac.ir
[2] Plant Protection Office, Agriculture Organization, Chaharmahal va Bakhtiari, Islamic Republic of Iran.
[3] Department of Plant Protection, Faculty of Agriculture, University of Shahrekord, Islamic Republic of Iran.

branches and trunks, weakening and sometimes killing them. The damage can be particularly extensive in the nurseries. Young trees of apple and olive, bearing a fruit load may be decayed because of the damage (Esmaili, 1991; Hegazi *et al.,* 2016). Among the fruit trees, the pest causes significant damage and yield loss on apple, pear, olive and walnut (Kutinkova *et al.,* 2006; Hegazi *et al.,* 2016; Saeidi, 2020). According to Kutinkova *et al.* (2006) more than 70% of stems were damaged by the pest in apple orchards and 30% of the young trees (three-year old) were perished in non-protected orchards in Bulgaria. Merghem and Ahmed (2017) reported the infestation level of *Z. pyrina* ranged from 15% to 74% and 1.2 to 4.4 holes/ tree in olive orchards in Egypt.

Pheromone traps were used to study seasonal flight and population dynamics of *Z. pyrina* on different host plants (Kutinkova *et al.,* 2009; Hegazi *et al.,* 2010, 2015; Kolyaee and Hasani, 2014; Ardeh *et al.,* 2014; Besharatnejad *et al.,* 2016; Suheri *et al.,* 2020; Saeidi, 2020). The main component of sex pheromone, identified from female pheromone glands, was (E,Z)-2,13-octadecadienyl acetate (Tonini *et al.,* 1986), but this compound attracts *Z. pyrina* males only when about 5% of (E,Z)-3,13-octadecadienyl acetate is added to it (Pasqualini and Natale, 1999; Kutinkova *et al.,* 2009).

Outbreak of *Z. pyrina* has occurred on the walnut trees in Saman Orchards, Chaharmahal va Bakhtiari Province, Iran, since 2006, and the pest attack is still increasing and spreading to other parts of the province. Thus, to apply effective control methods, knowledge of the biology and population dynamic of *Z. pyrina* is very important and necessary.

In this study, we aimed to monitor seasonal flight of the adults, larval activity, and damage caused by *Z. pyrina* in the walnut orchards, Chaharmahal va Bakhtiari Province, Iran. Results are useful to develop a successful integrated *Z. pyrina* management program.

MATERIALS AND METHODS

The Studied Area

The study was conducted during two successive years (2014-2015) in three regions infected by *Z. pyrina* in Chaharmahal va Bakhtiari Province, Iran, including Saman (latitude 32° 28” N, longitude 50° 54” E and height 1936 m), Shahre-Kord (Chaleshtor) (32° 23” N, 50° 48” E and 2110 m) and Farrokh-shahr (32° 18” N, 50° 01” E and 2169 m). This province is located in south-west of Iran, has a cold climate conditions, and is one of the regions in Iran famous for walnut plantation. Mean temperature and absolute minimum temperature of the studied areas are shown in Table 1. The studied orchards in Saman, Shahre-kord and Farokh-shahr cover about 20, 10 and 10 ha area, respectively. Trees were approximately 15-20 years old, with13-14 m height, and planted at 10×8 m distances between and along the rows. No chemical was applied on experimental plots during the period of study.

Pheromone, Trap and Installation Height

The pheromone dispensers, type of trap and installation height were followed according to Saeidi (2020). Delta traps (gray color, triangle-shaped houses, 21 × 18 cm in base and 11 cm in height) were made of plastic (2 mm diameter) (Nafis Cartonplast Ind., Iran). A sticky sheet (21 × 18 cm in length and width, respectively) was placed inside each trap. One pheromone dispenser of *Z. pyrina* (PH-990-1PR, Russell IPM Company, UK) was placed in the center of sticky sheet. The dispensers were polyethylene containers loaded each with 10 mg of *Z. pyrina* pheromone; 95% (E, Z)-2,13-octadecenyl acetate and 5% (E, Z)-3,13-octadecenyl acetate. Delta traps were installed at the height of 12 m above ground

Table 1. Mean temperature and absolute minimum temperature (AMT) in the studied orchards during 2014-2015.

Parameter	Station	Year	January	February	March	April	May	Jun	July	August	September	October	November	December	Annual
Mean temperature	Saman	2014	-2.7	1.5	7.3	13.4	17.5	22.8	26.2	24.7	21.6	15.0	5.7	5.1	13.2
Mean temperature	Saman	2015	5.1	5.7	7.9	14.0	18.6	24.2	24.4	24.0	19.9	17.0	8.7	1.6	14.3
Mean temperature	Farokhshahr	2014	-4.2	0.6	7.0	12.5	16.3	21.9	25.3	23.2	19.5	13.6	5.2	3.8	12.1
Mean temperature	Farokhshahr	2015	4.1	5.0	6.7	12.5	16.7	22.8	24.0	22.5	18.8	15.3	7.6	2.0	13.2
Mean temperature	Shahrekord	2014	-6.1	-0.7	5.7	11.2	14.9	20.3	24.0	21.8	17.7	12.2	4.1	2.3	10.6
Mean temperature	Shahrekord	2015	2.9	4.1	5.5	11.4	15.1	21.2	22.9	21.0	17.4	13.5	6.5	0.8	11.9
AMT	Saman	2014	-17.0	-14.6	-4.0	-0.6	6.8	9.8	15.8	11.5	10.8	3.6	-5.0	-6.2	0.9
AMT	Saman	2015	-7.0	-7.2	-9.9	-1.8	4.8	13.1	11.5	11.7	7.5	6.2	-3.6	-8.8	1.4
AMT	Farokhshahr	2014	-28.1	-16.5	-5.5	-2.9	4.3	4.6	12.4	9.1	5.4	-0.9	-11.4	-9.3	-3.2
AMT	Farokhshahr	2015	-10.9	-9.1	-7.5	-2.3	3.5	9.1	10.8	10	4.3	2.2	-7.1	-11.6	-0.7
AMT	Shahrekord	2014	-28.6	-22.1	-7.7	-5.2	0.5	1.6	9.0	5.8	1.6	-5.4	-15.8	-12.3	-6.6
AMT	Shahrekord	2015	-13.2	-12.6	-11.5	-4.1	-0.4	5.9	9.9	6.2	1.8	-1.8	-10.4	-15.3	-3.8

[a] AMT= Absolute minimum temperature.

level (about 1 meter below the apical point of tree canopy) on the outer edge of the tree canopy. For installation, a long cotton/polyester thread was tied to the trap, and then, it was hanged on a branch of tree with the help of a long stick. The lower part of the thread was attached to the tree trunk; therefore, the trap could be easily pulled up and down for recording the data or replacing lures and sticky sheets (Saeidi, 2020).

Seasonal Activity of the Pest

Seasonal flight of the adults was studied during 2014-2015 using pheromone traps. Four pheromone baited-traps were installed in each location, and to avoid interference between them, the distance between two adjacent traps was set at 50 meters (Ardeh *et al.*, 2014). All traps were set at a height of 1 meter below the apical point of the trees canopy and leaves and branches were removed around their entrances. The traps were set up in each location from May 10 (before the emergence of adult males) to July 15 (the end of the adults' flight) to monitor pest population. Pheromone traps were checked twice a week until the capture of the first adult, and after that, they were checked once a week and numbers of captured moths were recorded. The sticky sheets and the pheromone lures were replaced every two weeks and every month, respectively.

Observation on development of different stages of the pest (eggs, larvae and pupae) was done weekly throughout the growing season. In each location, 10 infested branches (one-year old) were observed weekly for presence of eggs and 1^{st} to 3^{rd} instar larvae, from June to September, whereas for 4^{th} and 5^{th} instar larvae and pupal stages, 10 active galleries (on trunk or main branches) were inspected from October to November and also from April to May.

Twig Infestation

Since the young larvae bore into twigs and feeds, the number of infested twigs per tree was determined in each orchard. This was performed during the second week of August, because activity of larvae was

maximal on the twigs at this time. For this purpose, 10 trees in each location were selected randomly and 10 random twigs from middle portion (at different sides) of each tree were examined. The infestation ratio was calculated using the following equation:

$$\text{Infested twigs (\%)} = \frac{\text{Number of infested twigs}}{\text{Total number of examined twigs}} \times 100$$

Number of Active Galleries per Tree

As the 4th and 5th instar larvae bore into trunk and the main branches, the number of active galleries/ tree was determined in each orchard during the second week of October. For this purpose, 10 trees in each location were selected randomly and the number of active galleries on the trunk was recorded up to 4 m height.

Statistical Analyses

Statistical analysis was performed using SAS program (SAS 9.1 Institute Inc) and SPSS (version 22) software. Proc GLM (general linear model) was performed to identify significant differences among the treatments and means were compared using LSD test at P=0.05. Data normality was assessed using Kolmogorov–Smirnov test and, data conversion was performed using the formula $\sqrt{x} + 1$, if required. The graphs were created using Excel software.

RESULTS

The studies conducted during 2014 to 2015 in three infested regions of Chaharmahal va Bakhtiari Province showed that the pest completed its life cycle within a year; in other words, the pest had one generation per year. Leopard moth overwintered as 4th and 5th instar larvae inside the galleries in trunk and main branches of walnut trees. In the first to second week of April, when the mean daily temperature increased to 10 ˚C (Table 1), the overwintered larvae started their feeding inside the galleries. The entry holes of the larvae are quite visible (on trunks and big branches) since masses of sawdust and frass protrude from them along with sap outflows. From the third week of April the fully grown larvae pupated in the feeding galleries. The pupal period completed within 10-14 days and adult started emerging from the second week of May, when the mean temperature increased to 15 ˚ C (Table 1). The first capture was observed from 12 to 18 of May in walnut orchards of the studied areas (Figures 1 and 2). Then, the population of *Z. pyrina* continuously increased and reached the maximum density on June 12 and June 6 in 2014 and 2015, respectively. At the peak flight of 2014, the numbers of trapped moths were recorded as 61.3 ± 3.33, 47 ± 2.50, and 90.30 ± 8.33 moths/trap/week (Figure 1), whereas in 2015, 56.60 ± 2.66, 52.60 ± 2.887, and 80± 5.60 moths/trap/week (Figure 2) were captured in Saman, Farokh-shahr and Shahre-kord (Chaleshtor), respectively. Thereafter, the number of captured moth decreased and within 3 to 4 weeks reached zero. The adult emergence lasted in the second decade of July (July 10 in Saman and July 16 in Chaleshtor orchards). According to the results, the flight period of *Z. pyrina* varied from 40 to 56 days in the studied areas and this pest had only one peak of flight in Chaharmahal and Bakhtiari Province.

Oviposition started 7-10 days after adult emergence, and eggs were laid individually on the branches next to the buds. After 10-14 days, eggs were hatched and neonate larvae entered directly into wood. *Z. pyrina* has five larval instars, in which the 1st, 2nd and 3rd instars feed inside the twigs and one-year old branches and usually tunnel downwards, whereas the 4th and 5th instars feed upwards within the main branches and trunk. While feeding from the twigs and young branches, the larvae may change their position several times and enter from

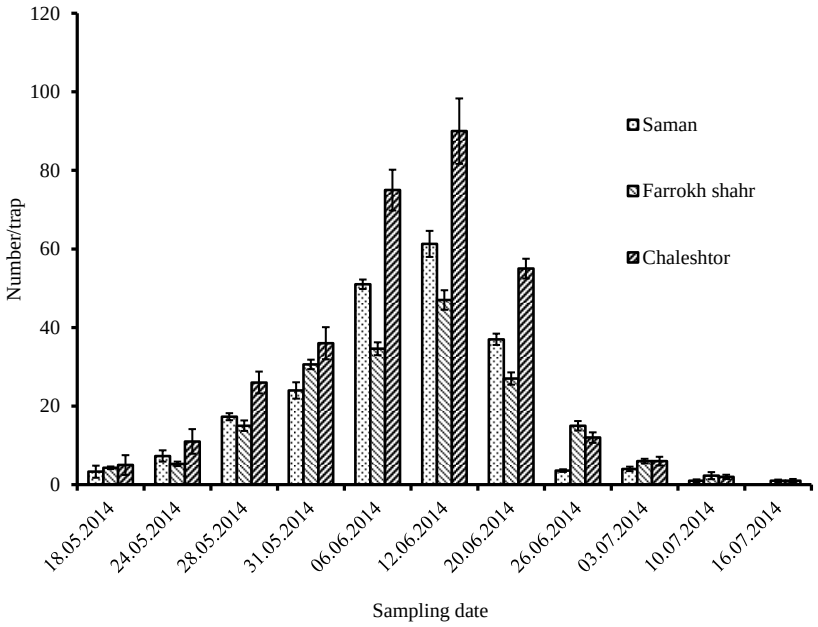

Figure 1. Seasonal flight of *Zeuzera pyrina* L. adults in different locations of Chaharmahal va Bakhtiari Province, in 2014.

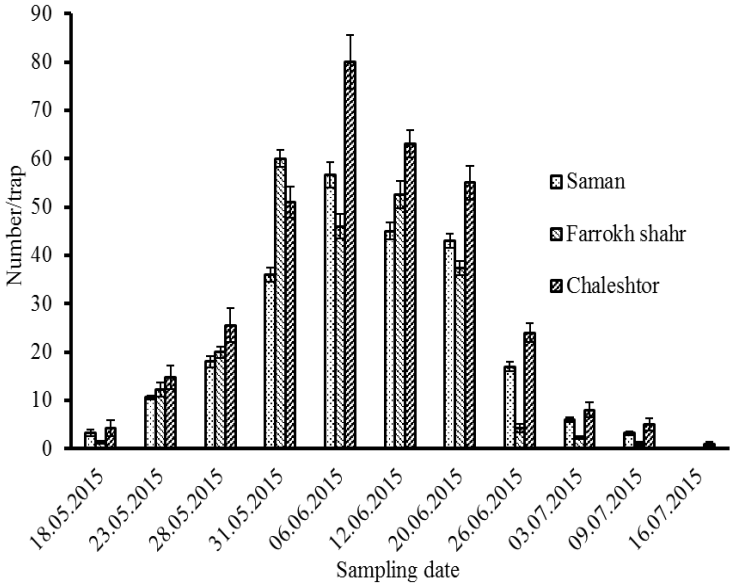

Figure 2. Seasonal flight of *Zeuzera pyrina* L. adults in different locations of Chaharmahal va Bakhtiari Province, in 2015.

another place. The infested twigs dry out and the fruits attached to them become wrinkled and unusable. The maximum activity of larvae on the twigs was observed during August; therefore, pruning of the infested twigs is an effective method to reduce the pest population. From late September, the 4th instar larvae gradually moved to the lower parts of the tree and made their galleries upward on the main branches and tree trunk. Due to the increased feeding activity of the larvae, a

large amount of plant sap along with the larval feces secreted from the active galleries. The feeding activity of larvae was observed till mid-November, when the mean daily temperature was between 5 to 8 ℃ (Table 1), then, it was stopped and the larvae overwintered until the next spring.

Study on percentage of infested twigs/ tree by *Z. pyrina* in different locations revealed that the pest caused heavy damage to walnut twigs in the three studied locations. In 2014, mean damage to walnut twigs were calculated as 56 ± 2.62, 50 ± 2.36, and 79 ± 3.09% in Saman, Farrok-shahr and Chaleshtor orchards, respectively, whereas, in 2015, the pest infested 60.33 ± 2.13, 55.66 ± 2.84, and 85 ± 2.36% of walnut twigs in the studied locations, respectively (Figure 3).

The highest number of active galleries was observed in Chaleshtor (28 ± 2.49 and 42.30 ± 3.54) and the lowest in Farrok-shahr (21 ± 2.16 and 21.33 ± 1.44 galleries/tree) in 2014 and 2015, respectively. The numbers of active galleries/tree in Saman orchards were recorded as 18.33 ± 2.32 and 25.67 ± 4.18, which were not significantly different from Chaleshtor (Figure 4).

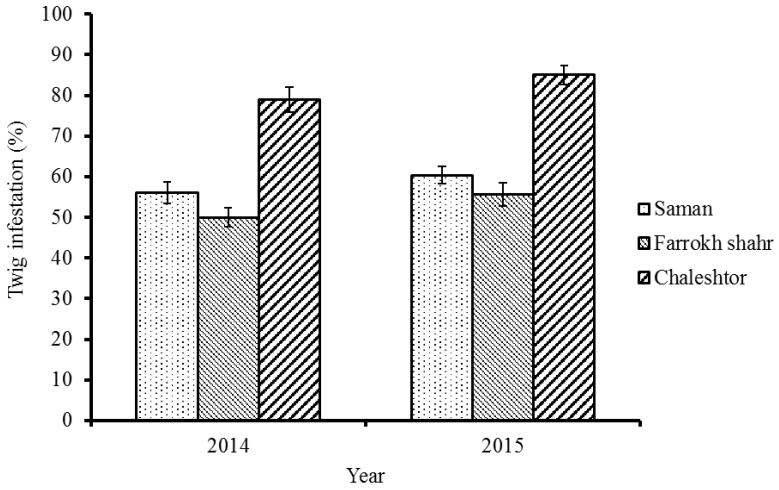

Figure 3. The infestation ratio of walnut twigs caused by *Zeuzera pyrina* L. larvae in different locations of Chaharmahal va Bakhtiari Province, during 2014–2015.

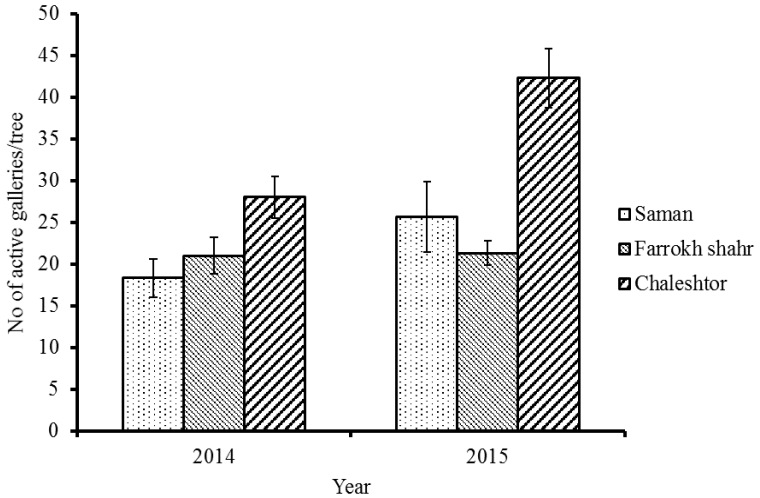

Figure 4. Number of active galleries/tree caused by *Zeuzera pyrina* L. larvae in different locations of Chaharmahal va Bakhtiari Province, during 2014–2015.

DISCUSSION

Leopard moth has become a serious pest of walnut in Chaharmahal va Bakhtiari since 2006. Heavy damage of the pest was first observed in Hossain Abad Orchard, Saman, and spread rapidly in other parts of the province. In this paper, we discuss the differences observed in biology and life cycle of *Z. pyrina* with earlier reports in Iran and other parts of the world.

In the current study, to determine the seasonal flight, the pheromone dispenser of *Z. pyrina* was used according to Saeidi (2020). He reported that *Z. pyrina* pheromone dispenser captured significantly more *Z. pyrina* males and was more effective, compared to *Synanthedon tipuliformis* (Clerck) pheromone dispenser, therefore, it was used in this study. Delta trap used in this study was reported as an effective trap -body type for capturing of *Z. pyrina* males by different researchers (Pasqualini and Natale, 1999; Hegazi *et al.*, 2009; Ardeh *et al.*, 2014; Besharatnejad *et al.*, 2016; Saeidi, 2020). The traps were installed at the height of 12 m above ground level (one meter below the apical point of trees canopy). According to Saeidi (2020), the number of trapped moth at this height in the walnut orchards (Saman, Chaharmahal va Bakhtiari Province, Iran) was 11.6 and 6.59 times greater than those installed at 4 and 6 meters above the ground, respectively.

Our results indicated that the life cycle of *Z. pyrina* was completed within a year in Chaharmahal va Bakhtiari Province. According to other researchers, some individuals complete their life cycle in a year but others may require two years to complete their development (Radjabi, 2002; Kutinkova *et al.*, 2009; Hegazi *et al.*, 2015). Reports from different provinces of Iran such as Alborz (Esmaili, 1991; Radjabi, 2002; Kolyaee and Hassani, 2014), Markazi (Radjabi, 2002) and Esfahan (Radjabi, 2002; Besharatnejad *et al.*, 2016) showed that for those individuals that complete their life cycle in one year, larvae overwintered as mature or fourth instars, whereas for those which develop over two years, the first or second instars larvae overwintered in the first winter. In addition, it was reported that *Z. pyrina* required one or two years to complete its life cycle in olive orchards in Egypt (Hegazi *et al.*, 2015; Merghem and Ahmad 2017) and apple orchards in Syria (Almanoufi *et al.*, 2012) and Bulgaria (Kutinkova *et al.*, 2009). In another study, Zohdi and Kolyaee (2016) showed that *Z. pyrina* required two years to complete one generation in walnut orchards of Kerman Province, Iran.

The flight season of adult *Z. pyrina* usually begins in the second decade of May in the studied areas, with peak flight occurring in the first decade of June, and usually ending by second decade of July. Therefore, emergence of adults occurs in a short period of time with only one peak in June, in contrast with findings of other researchers. Hegazi *et al.* (2015) reported that, in olive orchards of Egypt, moth emergence occurred from late April to mid-November, with two peaks (minor peak in May and Major peak in September) in most years. However, Radjabi (2002) reported that emergence of adults in walnut and apple orchards in Karaj (Iran) occurred from May to September, with major peak in June and minor peak in September. In another study, Kutinkova *et al.* (2006) reported that in apple orchards of Bulgaria, the moth appeared from 1st to 3rd decade of June and flight continued till mid-September.

Our observations have shown that most eggs were laid individually near the buds on the twigs and 1st instar larvae tunneled directly into inner wood. Similar observation was reported by Zohdi and Kolyaee (2016) in walnut orchards of Kerman Province, Iran, whereas Radjabi (2002) and Sarto-Monteys (2015) reported that females had heavy-bodies and often laid their eggs in clusters mostly on the trunk near the sites where they emerged from pupae. In that case, the 1st instar larvae disperse to bore into the tips of branches and shoots. We

found that the pest overwintered as 4th and 5th instar larvae, whereas Esmaili (1991), Radjabi (2002), and Hegazi *et al.* (2009) believed that different instars remained in a dormant stage for overwintering and young larvae continued their feeding for the next year.

The maximum damage to shoots and twigs of walnut trees was observed at the third decade of August and thereafter larvae gradually moved to the main branches and trunk. Kutinkova *et al.* (2006) reported the maximum of damage to apple shoots in Bulgaria occurred at the end of August and beginning of September. Merghem and Ahmed (2017) recorded the highest levels of infestation (74%) in olive orchards of Egypt, which was similar to our finding in walnut orchards of Shahre-kord (79% and 85% in 2014 and 2015, respectively). We observed that *Z. pyrina* could successfully infest both healthy and stressed walnut trees in Chaharmahal va Bakhtiari Province, in contrast with the earlier reports that healthy plants are resistant to the borer, except during periods of environmental stress such as prolonged drought (Radjabi, 2002). Many researchers believed that woodborers rarely infest healthy plants and their larvae may be killed by sap flow (Esmaili, 1991; Radjabi, 2002; Van Driesche and Reardon, 2015).

Results of this study showed that biology and seasonal flight pattern of *Z. pyrina* in Chaharmahal va Bakhtiari Province is different from other regions and that may be because of environmental condition in this province and adaptation of *Z. pyrina* population to these conditions. According to Khaliq *et al.* (2014), the climatic and weather changes not only affect the status of insect pests but also affect their population dynamics, distribution, abundance, intensity and feeding behavior. Since Chaharmahal va Bakhtiari is one of the coldest region in Iran, those larvae that cannot develop to the 4th instar and cannot migrate to thick branches before December may die during the cold winter. Therefore, over a period of time, the individuals that complete their life cycle within a year were selected. According to meteorological data (Table 1), the absolute minimum temperature falls below -20 ℃ in the studied areas during January and February, which may kill the young larvae in the soft, thin and none-woody branches.

Knowledge of the observed differences may be useful in making decision for management of *Z. pyrina*, specially using pheromone traps (for monitoring, mass trapping or mating disruption) and other techniques (such as removal of infested branches) for decreasing damage and population density of the pest. Of course, further investigations are suggested to determine the effect of different climatic factors on population dynamics and damage of *Z. pyrina* in walnut orchards.

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فعالیت فصلی و خسارت ناشی از پروانه فری، *Zeuzera pyrina* L.، در باغ‌های گردو، استان چهارمحال و بختیاری ، ایران

ز. سعیدی، ع. باقری، و ا. خلیلی مقدم

چکیده

در این مطالعه پرواز فصلی حشرات کامل، فعالیت لارو و خسارت ناشی از آفت *Z. pyrina* در باغ های گردو، استان چهارمحال و بختیاری، بررسی شد. تله‌های فرمونی از 20 اردیبهشت (قبل از ظهور حشرات بالغ) تا 25 تیرماه (پایان پرواز) در باغ‌های مورد مطالعه نصب شدند. مشاهدات مربوط به مراحل مختلف رشدی آفت به صورت هفتگی در طول فصل رشد انجام گردید. میزان آلودگی شاخه‌ها و تعداد دالان‌های فعال روی هر درخت در مکان‌های مورد مطالعه ثبت شد. نتایج نشان داد که آفت چرخه زندگی خود را در مدت یک سال به پایان رسانده و به صورت لارو سن 4 و 5 در داخل دالان در شاخه های اصلی و تنه درختان گردو زمستان گذرانی می‌کند. دوره پرواز حشرات کامل *Z. pyrina* از دهه سوم اردیبهشت ماه آغاز و در دهه دوم خردادماه به اوج رسیده و دردهه سوم تیرماه پایان می‌یابد. تخم‌ها بطور انفرادی در کنار جوانه ها و روی سرشاخه‌ها گذاشته می‌شوند و لاروهای سن اول مستقیماً به داخل چوب نفوذ می‌کنند. بیشترین خسارت به سرشاخه ها در اوایل شهریورماه مشاهده و بعد از آن لاروها به تدریج به شاخه‌های اصلی و تنه منتقل می‌شوند. نتایج این مطالعه نشان داد که زیست شناسی و الگوی پرواز فصلی *Z. pyrina* در این استان با سایر مناطق آلوده متفاوت است که ممکن است به دلیل شرایط آب و هوایی منطقه باشد. آگاهی از اختلافات مشاهده شده ممکن است در تدوین یک برنامه موفق مدیریت تلفیقی برای کنترل کرم خراط مهم باشد.