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Screening walnut and apple trees against Leopard moth, *Zeuzera pyrina* (Lep.: Cossidae)

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Abstract

Leopard moth, *Zeuzera pyrina* L. (Lep.: Cossidae), is a xylophagous pest which feeds on several host plants such as apple, pear, walnut and quince trees. Its activity results in withered branches and yield loss, finally causing death of trees. Detecting resistance mechanisms in resistant trees and deploying resistant varieties can be useful in managing leopard moth and conserving the environment from detrimental side effects of pesticides. In this research, resistance of 9 walnut and 17 apple varieties in orchard collection of Seedling and Seed Research Institute (Kamal-Shahr, Alborz province) was studied during 2009-2010 based on a completely randomized block design. For walnut varieties, 6 cohort trees for each variety were chosen and the number of larval entrance holes in six branches (four outside and two inside of canopy)/tree, were studied. For apple varieties, 3 cohort trees for each variety were chosen and the amount of larval feces at the base of each apple tree (based on the amount of larval fecal pellets which can be held in a tablespoon) and the number of larval entrance holes from base of the apple trees up to 1.5 m-height were studied. The maximum and minimum number of larval entrance holes in walnut varieties were observed on 'Z30' (6.16±0.45) and 'Z63' (1.25±0.49) respectively. Therefore, 'Z63' is recommended for further study regarding the detection of resistance mechanisms. In apple varieties, the maximum number of larval entrance holes (11.0±3.1) and maximum amount of larval feces (7.0±1.3 tablespoons) were recorded on 'Richard Delicious'. 'Northern spy', 'Jonathan', 'Prime Gold' and 'Red spur cooper' varieties received the least damage (near zero) compared to the other varieties and they are recommended for further studies.
Key words: resistance, apple, walnut, Leopard moth, varieties, *Zeuzera pyrina*, mechanism.

بررسی میزان آلودگی ارقام مختلف گردو و سیب به کرم خراط، *Zeuzera pyrina* (Lep.: Cossidae)

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چکیده

کرم خراط، *Zeuzera pyrina* L. (Lep.: Cossidae)، آفتی چوب‌خوار با میزبان‌های سیب، گلابی، گردو و به است. فعالیت آفت منجر به خشک شدن شاخه‌ها، کاهش محصول و در نهایت مرگ درخت می‌شود. تعیین مکانیسم‌های مقاومت و استفاده از واریته‌های مقاوم علیه این آفت می‌تواند در مدیریت آن و نیز حفظ محیط زیست از اثرات سوء سموم، مفید باشد. در این تحقیق، آلودگی ۹ واریته گردو و ۱۷ واریته سیب باغ کلکسیون موسسه تحقیقات نهال و بذر (البرز- کمال شهر) از نظر تفاوت در میزان خسارت این آفت در قالب طرح بلوک‌های کامل تصادفی (۱۳۸۹-۱۳۹۰) بررسی شد. در واریته‌های گردو، هر ژنوتیپ دارای شش اصله درخت هم سن بود. در هر درخت گردو، چهار شاخه ۷۰-۶۰ سانتی‌متری از چهار جهت مختلف و دو شاخه از داخل کانوپی انتخاب شده و سوراخ‌های جدیدی که در اثر نفوذ لاروهای همان سال ایجاد شده بودند در طول شاخه‌های مذکور شمارش و ثبت گردد. در واریته‌های سیب، هر ژنوتیپ دارای ۳ اصله درخت هم سن بود. میزان فضولات ریخته شده در زیر هر درخت سیب (تخمین بر مبنای میزان فضولاتی که در یک قاشق غذاخوری جا می‌شد) و تعداد سوراخ‌های فعال روی تنه درختان سیب از سطح زمین تا ارتفاع ۱/۵ متری بررسی شد. حداکثر تعداد تونل‌های لاروی در واریته‌های گردو در رقم'Z30' (۶/۱۶±۰/۴۵) و حداقل تعداد تونل‌های لاروی در رقم 'Z63' (۱/۲۵±۰/۴۹) مشاهده شد. رقم گردو 'Z63' به دلیل داشتن خسارت کم، جهت مطالعه تکمیلی مکانیسم‌های مقاومت توصیه می‌گردد. در واریته‌های سیب، حداکثر تعداد تونل‌های لاروی (۱۱/۰±۳/۱) و حداکثر میزان فضولات لاروی (۷/۰±۱/۳ قاشق غذاخوری) در واریته سیب 'Richard Delicious' مشاهده شد. میزان فضولات لاروی و تعداد تونل‌های لاروی در ارقام سیب 'Northern spy'، 'Jonathan'، 'Prime Gold' و 'Red spur cooper' بر اساس بررسی‌های دو ساله برابر با صفر بوده و جهت مطالعه تکمیلی توصیه می‌گردند.
واژه‌های کلیدی: آلودگی، گردو، سیب، کرم خراط، *Zeuzera pyrina*، مکانیسم.

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Introduction

Walnut is an important economic fruit tree in Iran due to its high nutritional value. The main walnut varieties in Iran are 'Franket', 'Lara', 'Pedro', 'Hartley', Serr', Viena', Chandler', 'Z67', 'Z63', 'Z30', 'Z60', 'B63/1' and 'b4/73/2'. The main walnut orchards are in Kerman province followed by Hamedan, Ghavin, East-Azarbayjan, Kermanshah, Northern Khorasan and Fars provinces (Anonymous, 2012). Apple is an important economic orchard crop in Iran due to its high amount of A, B and C vitamins and also potassium, calcium, sodium, iron and phosphor. There are about 160,000 ha of apple orchards in Iran. West-Azarbayjan province has the highest area of apple orchards (55,000 ha). The common apple verities grown in Iran are as follows: 'Golab', Shafiabadi', 'Ghandak', 'Abbas Mashad', 'Golshani', 'Shemirani', 'Zonooz', 'Red & Golden Delicious', 'Jonathan', 'Starking', 'Granny Smith', 'Starcrimson', 'Redstar' and 'Fuji' (Anonymous, 2012). Leopard moth, *Zeuzera pyrina* L. (Lep.: Cossidae), has become an important pest of walnut in Iran, since the end of 1980s during which several drought periods occurred. Its main habitat is Europe (Behdad, 1997). It was reported from Iran in 1949 by Davachi. Rajabi (1976) observed it in Markazi, Zanjan, Gorgan, Gilan, Mazandaran and Khorasan provinces. The first study on its biology and ecology in Iran was done by Rabieh (1963). Leopard moth attacks walnut, apple, quince, elm and chestnut trees (Rajabi, 1976). The main damage occurs at phloem and xylem vessels of trees. The signs of damage include dark fluid along with larval orange feces coming out of the larval tunnels. Larval tunnels can reach up to 50 cm. Damage results in tree weakness, attraction of bark beetles and finally tree death (Rajabi, 1976). At warm environmental condition, the damage rate is high (Esmaili, 1996). Leopard moth has two generations in three years in Kamal-Shahr area. It hibernates as larvae inside main branches or stems of trees. In the middle of the spring, the last instar larvae, pupate and after two weeks adults emerge. In Kamal-Shahr (Alborz, Iran), adults' emergence period is from June until September. The biology and behavior of this pest has been studied by several researchers (Rajabi and Daniali, 1969; Talhouk, 1969; Esmaili, 1996; Behdad, 1997). Application of paste insecticides inside the larval tunnels and mass trapping by sex pheromone have been reported as the effective control methods against Leopard moth (Solomon, 1995). From practical point of view, deployment of resistant varieties is easier than insecticide application (Smith, 1989). Several studies have been conducted on resistance of trees against pests, such as deployment of resistant spruce varieties against xylophagous beetles in China, and deployment of pine resistant varieties to gall midge, *Thecodiplosis japonensis* (Dip.: Cecidomyiidae) in south Korea and Japan (Wagner *et al.* 2002). Study on resistance of hawthorn varieties against hawthorn moth, *Holcocerus hippophaecolus* (Lep.: Cossidae), in China, revealed that its oviposition rate was different among varieties due to anixenosis resistance. The susceptible varieties emitted volatiles which attracted the moths. Hawthorn trees characteristics such as size of stomata, density of vessels and bark roughness were not correlated with resistance level. However, the wood compactness of these varieties was correlated with resistance level (Zhizheng, 2010). The goal of this research was to study resistance of different apple and walnut varieties, grown in Seed and Seedling Research Institute (KamalShahr, Alborz, Iran) orchard, to Leopard moth during 2010-2011. The results could be useful on developing resistant varieties to the Leopard moth.

Materials and Methods

In orchard of Seed and Seedling Research Institute (KamalShahr, Alborz, Iran) (35.855361° N, 50.864006° E), 9 walnut varieties ('Z30', 'R.P.M.', 'Serr', 'Hartley', 'Chandler', 'Pedro', 'K72', 'Z63', 'Z53') and the following 17 apple varieties were studied (origins are mentioned in parentheses): 'Richard Delicious' (U.S.A), 'Mashhad-e Nouri' (Iran), 'Sheikh Ahmad' (Iran), 'Haji-e Karaj' (Iran), 'Belle de pontoise' (France), 'Red Rome Beauty' (U.S.A), 'Yellow transparent' (Russia), 'Ardebil' (Afghanistan), 'Golab-e sahne' (Iran), 'GoldJon' (Italy), 'Golab-e Isfahan' (Iran), 'Red Delicious' (U.S.A), 'Nayan-e Aranghe' (Iran), 'Jonathan' (U.S.A), 'Prime Gold' (U.S.A), 'Cooper Red Delicious Spur' (U.S.A) and 'Northern Spy' (U.S.A). Study was done based on a completely randomized block design. Each walnut and

apple variety had six and three cohort trees (replications), respectively. For walnut trees, 4 branches (1 meter in length) from outside of the canopy and two branches (1 meter in length) from inside of the canopy were selected in each replication and the number of active larval tunnels was counted on 1 September 2010 and 4 September 2011. For apple trees, the number of active larval tunnels (from base of the trees up to height of 1.5 m) and the amount of larval feces which can be held in a tablespoon were recorded on 1 September 2010 and 4 September 2011. The active tunnels could be recognized by the tree sap coming out of the tunnels and the feces attached to this fresh sap (fig. 1).

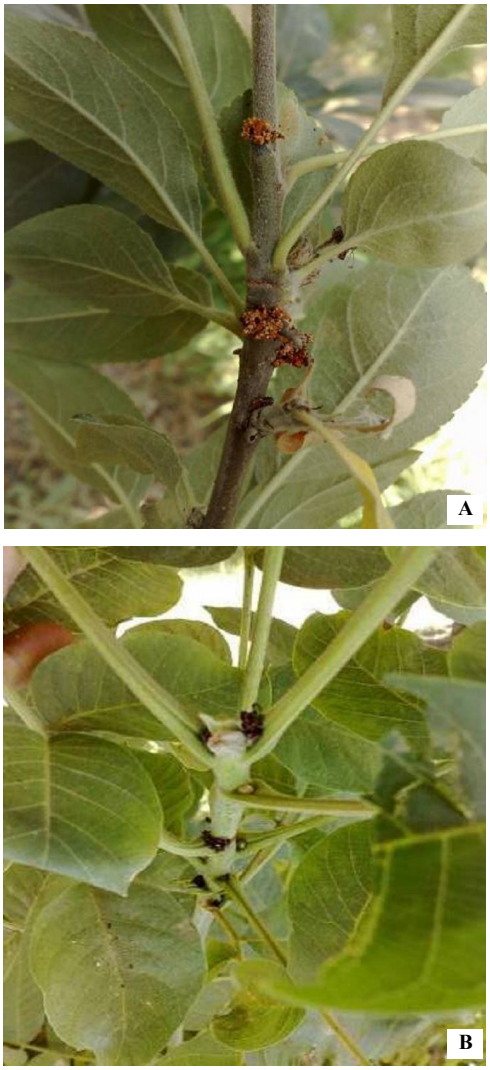

Fig. 1. Apple (A) and walnut (B) twigs infested with leopard moth larvae

A mixed model analysis of variance (PROC MIXED) was used to compare the number of larval entrance and larval feces for each treatment. The Satterthwaite method (Satterthwaite, 1946) was applied for determining the denominator degrees of freedom (d.f.). Data were log transformed. Comparisons among the treatments were made using the Tukey-Kramer test where analysis of variance showed significant differences among means. Correlation between larval feces and number of tunnels was estimated (150 points) (PROC CORR) (Saxton 1998, SAS Institute Inc., 2002).

Results and Discussion

The number of larval tunnels and also the amount of larval feces were not different between 2010 and 2011 among apple varieties (MIXED ANOVA: $F_{16,66}$ = 0, P = 1), therefore, the data were pooled. The number of larval tunnels (MIXED ANOVA: $F_{16,80}$ = 10.250, P < 0.0001 and the amount of larval feces (MIXED ANOVA: $F_{16,80}$ = 10.90, P = <0.0001) were significantly different among apple varieties (Table 1). There was a significant positive correlation between larval feces and number of larval tunnels (r_s = 0.86, P <0.0001).

The number of larval tunnels and also the amount of larval feces were not different between 2010 and 2011 among walnut varieties (MIXED ANOVA: $F_{8,88}$ = 0, P = 1), therefore, the data were pooled. The number of larval tunnels (MIXED ANOVA: $F_{8,80}$ = 11.76, P <0.0001 were significantly different among the walnut varieties (Table 2) indicating difference in resistance level among the treatments. It is possible that there are some compounds in resistant lines that reduce larval growth and finally results in larval tunnel reduction. Difference among treatments might be related to difference in oviposition rate of females on these varieties as it was proved for hawthorn moth, *H. hippophaecolus* in China. The volatiles of varieties might affect the host finding behaviour of leopard moth, similar to that was observed for *H. hippophaecolus* (Zhizheng, 2010). Sometimes, the chemical compounds at sub-lethal doses increase the feeding rate of larvae, resulting in more damage in the resistant lines compared to susceptible ones (Winterer and Bergelson, 2001).

Table 1. The Mean (±SE) number of larval tunnels on the bark and mean (±SE) of larval feces at the base of 17 apple varieties, screened for resistance to leopard moth, *Zeuzera pyrina* L., at Kamalshahr (Alborz, Iran).

Variety	Mean (±SE) number of larval tunnels *	Mean (±SE) number of larval feces *‡
'Richard Delicious'	11.0±3.0 a	7.0±1.3 a
'Mashhad-e Nouri'	7.0±1.3 ab	3.3±1.1 a-c
'Sheikh Ahmad'	7.0±1.5 a-c	2.0±0.5 a-d
'Haji-e Karaj'	7.4±2.0 a-c	2.7±0.4 a-c
'Belle de pontoise'	6.0±1.8 a-c	3.1±0.9 a-c
'Red Rome Beauty'	5.4±1.5 a-c	4.3±1.8 ab
'Yellow transparent'	7.0±2.4 a-d	2.6±0.8 a-e
'Ardebil'	3.4±0.4 a-e	0.7±0.4 c-f
'Golab-e sahne'	5.0±2.0 a-e	1.7±0.6 b-f
'GoldJon'	3.3±1.3 a-f	1.7±0.7 b-f
'Golab-e Isfahan'	3.3±1.8 b-f	0.4±0.2 d-f
'Red Delicious'	1.7±0.8 c-f	0.4±0.2 d-f
'Nayan-e Aranghe'	1.0±0.6 d-f	0.2±0.1 ef
'Jonathan'	0.3±0.2 ef	0 f
'Prime Gold'	0 f	0 f
'Cooper Red Delicious Spur'	0 f	0 f
'Northern Spy'	0 f	0 f

* Means followed by same letters are not significantly different (Tukey-Kramer test, $P < 0.5$).

‡ The amount of larval feces which can be held in a tablespoon.

Table 2. The Mean (±SE) number of larval tunnels on the bark of 9 walnut varieties, screened for resistance to leopard moth, *Zeuzera pyrina* L., at Kamalshahr (Alborz, Iran)

Variety	Mean (±SE) number of larval tunnels*
'Z30'	6.16±0.45 a
'R.P.M.'	3.91±0.39 b
'Serr'	3.75±0.42 b
'Hartley'	3.83±0.60 b
'Chandler'	3.75±0.35 b
'Pedro'	4.16±0.50 b
'K72'	4.33±0.41 b
'Z53'	3.75±0.35 b
'Z63'	1.25±0.49 c

* Means followed by the same letters are not significantly different (Tukey-Kramer test, $P < 0.5$).

The chemical compounds of trees have substantial effects on larval activity. For example, lignin of conifers phloem can make 'stone cell mass' which can reduce the activity of bark beetle, *Dendroctonus micans* (Col.: Scolytidae) (Wainhouse *et al*. 1990; 1998). Similar mechanisms might be present in the studied varieties. While studying the antibiosis of varieties against leopard moth, two important factors which should be considered are soil humidity level and irrigation rate. Because they can stimulate the process of antibiotics production in a plant and also can change the ratio of chemical compounds. Based on a 6-year study in China on *H. hippophaecolus* (Zhou *et al*. 2007), it was proved that soil humidity level compared to soil fertility was more important in conferring hawthorn resistance against this pest. They found that increase in soil humidity together with increase in soil fertility result in photosynthesis and C/N ratio increase, change in Na/K balance and decrease in hawthorn trees temperature. Based on personal observation of the authors, the damage of leopard moth is less in well-irrigated orchards compared to trees which are under drought stress. In our study, 'Z63' walnut variety showed the least damage, and 'Northern spy' ،'Jonathan' ،'Prime Gold' and 'Red spur cooper' apple varieties did not show any sign of damage; therefore, they may have resistance mechanisms and need to be included in further studies. They may have compact wood as it was proved for resistance of hawthorn lines against *H. hippophaecolus* (Zhizheng, 2010). These lines need to be studied more and can be used in developing of resistant lines/varieties against leopard moth.

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