*Article*

# Response of adult male *Zeuzera pyrina* (Lep: Zeuzeridae) to different pheromone traps in walnut orchards of four isolated regions of Iran

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**Abstract**
The leopard moth, *Zeuzera pyrina* L. (Lep: Zeuzeridae) is an important polyphagous pest of a wide range of trees and shrubs across Europe and Asia. The efficient control of the leopard moth by direct spraying of infested branches and trees is usually impractical because the larvae feed internally and escape from exposure to the used insecticides. Sexual pheromones emitted by females may provide an efficient alternative method to explore the pest, disrupt its mating or capture and kill it. In the present study, we evaluated the efficiency of one Iranian-formulated (in three doses: 1, 1.5, and 2 mg per trap) and three non-Iranian-formulated sexual pheromones in attraction and capturing of adult males of the leopard moth in Walnut orchards of Alborz province and three different regions of Kerman province (Rabor, Baft, Darremorid). We found a wide diversity in response of adult males to different pheromones in the four studied regions. This diversity seems to be resulted from isolation of the populations and may present a preliminary stage of divergence and speciation in this species.

**Keywords** *Zeuzera pyrina*; adult male; sexual pheromones; traps; walnut orchards.

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## 1 Introduction

A large number of insect species are well known to use pheromones to attract mate during mating season (Roelofs et al., 2002; Witzgall et al., 2008; Shirangi et al., 2009). Since the discovery of the first pheromone (Butenandt et al., 1961), and the increased awareness of the harmful effects of conventional pesticides (Carlson, 1962), many researchers have focused on and fund to alternative methods of pest control (Carde, 1976). As one of these alternatives, a large volume of research programs have been directed toward elucidation of the chemical identity and behavioral role of pheromones associated with economically important species in the expectation that a synthetic pheromone could be re-arranged and used in pest control systems (Carde et al., 1975; Carde, 1976; Stelinski et al., 2009).

The leopard moth, *Zeuzera pyrina* L. (Lep: Zeuzeridae) is an important polyphagous pest of a wide range

of trees and shrubs, including apple, pear, quince, cherry, plum, grapevine, and walnut across Europe and Asia including Iran. The larvae of leopard moth feed inside the stems and branches of young trees causing die-backing of shoots, whitening of leaves and in main young stems, killing of the whole tree (Alford, 2007). The efficient control of the leopard moth by direct spraying of infested branches and trees is usually impractical because the larvae feed internally and escape from exposure to the used insecticides. This request the need for alternative control strategies among them, sexual pheromones potentially provide some perspectives.

Emitted by females, sexual pheromones are detected by male moth and help them to locate the calling females and mate with them. Both natural and synthetic pheromones of moths and many other insect species have been repeatedly used for control of economically important species via mating disruption, mass trapping and population evaluation (El-Sayed et al., 2006; Laurent and Frerot, 2007; Vacas et al., 2009; Isaacs et al., 2012).

In this study, the efficiency of six synthetic pheromones was evaluated on the attractiveness of adult males of the leopard moth in four different geographic regions of Iran. Our main goal was to clarify if different populations show any difference in their response to sexual pheromones produced by various companies across the world and to select the most attractive compound for control of this injurious pest in each studied region.

## 2 Materials and Methods

### 2.1 Study area

During 2009-2011, two sites in Alborz province (located at Kamalshahr and Nazarabad) and three sites in Kerman province (located at Baft, Rabor, and Darremorid) were selected. The selected sites in Kerman province were among the most important regions for production of walnut. The studied region in Alborz province is of much importance because it is among the main sites contributing to the production and distribution of walnut sapling to whole the country. Kerman and Alborz provinces are located at southern east (53º26´ to 59º29´E and 25º55´ to 32º N) and center of Iran (51º25´ to 50º14´ E and 36º20´ to 35º40´ N), respectively (Fig. 1). The precise locations of the studied sites are shown in Fig. 1. The average annual precipitations of Kerman and Alborz provinces are 350-400 and more than 400 mm, respectively. The minimum and maximum monthly temperatures of Kerman province are 7 and 39, while those of Alborz province are 1ºC (January) and 30ºC (August). Our studied areas include three different regions in Kerman province (Darremorid village, Baft county, and Tazraj village) and one region in Alborz province (Kamalshahr) (Fig. 1).

**Fig. 1** Geographical locality of Kerman and Alborz provinces, Iran.

### 2.2 Establishment of pheromone traps

In each aforementioned area, six Delta traps each contain one of the following sexual pheromones was set: leopard moth sexual pheromone formulated by the Iranian Plant Protection Institute in three different doses (0.5, 1, and 2 mg per trap), leopard moth sexual pheromones formulated by Russel (Canada) and AgriScience (Germany) corporations, and the sexual pheromone of the Sessiid moth formulated by Russel corporation. This experiment was conducted in three replicates so that a total numbers of 18 Delta traps were set in each studied region. All traps were established in a height of 120-180 cm and the distances between two traps were at least 50 m. The pheromone sources and the trap glues were refreshed every 45 days. All traps were established in early May and observations, and counting and deletion of captured moths were conducted within a five-month period by the end of September. Data of this study were analyzed by use of SAS computer software. Mean values were compared using Duncan test.

## 3 Results

Our results revealed that the appearance of adult leopard moths is different between the studied areas. The first male moth was captured at May and June in Kerman and Alborz provinces, respectively. The most adult males captured by the traps were related to *S. tipuliformis* sexual pheromone formulated by Russel Corporation (Canada) in Baft region (Fig. 2b), the leopard moth pheromone formulated by AgriScience Corporation (Germany) in Alborz province (Fig. 2a) and Rabor region (Fig. 2d), and the leopard moth pheromone formulated by Russel Corporation (Canada) in Darremorid region (Fig. 2c). The efficiency of the six sexual pheromones was individually compared within each studied area. The results revealed a significant difference between the capturing rate in Alborz province and Rabor region (One-way ANOVA, $F=11.5$, $P<0.01$ and $F=4.12$, $P<0.01$) (Fig. 2a and 2d). The efficiency of these pheromones, however, was not statistically different in Baft and Darremorid regions (One-way ANOVA, $F=1.03$, $P>0.05$; $F=1.99$, $P>0.05$; and $F=2.12$, $P>0.05$ for Baft, Darremorid, and Rabor, respectively) (Fig. 2b and 2c).

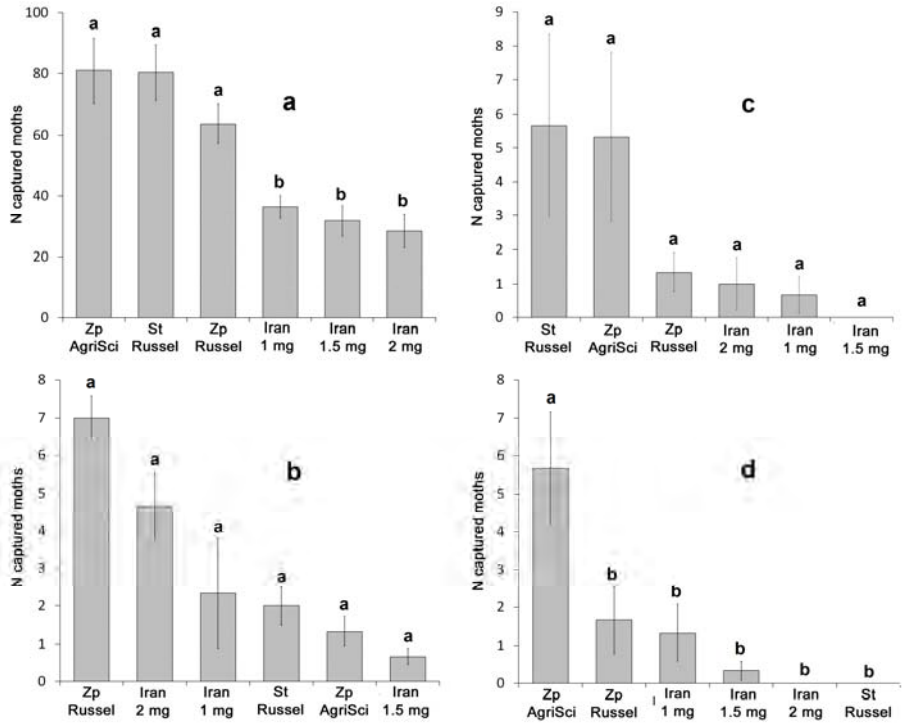


**Fig. 2** the average numbers of adult leopard moths captured by six commercially available sexual pheromones in four geographical region of Iran; a, Alborz province; b, Baft region, c, Darremorid; d, Rabor region.

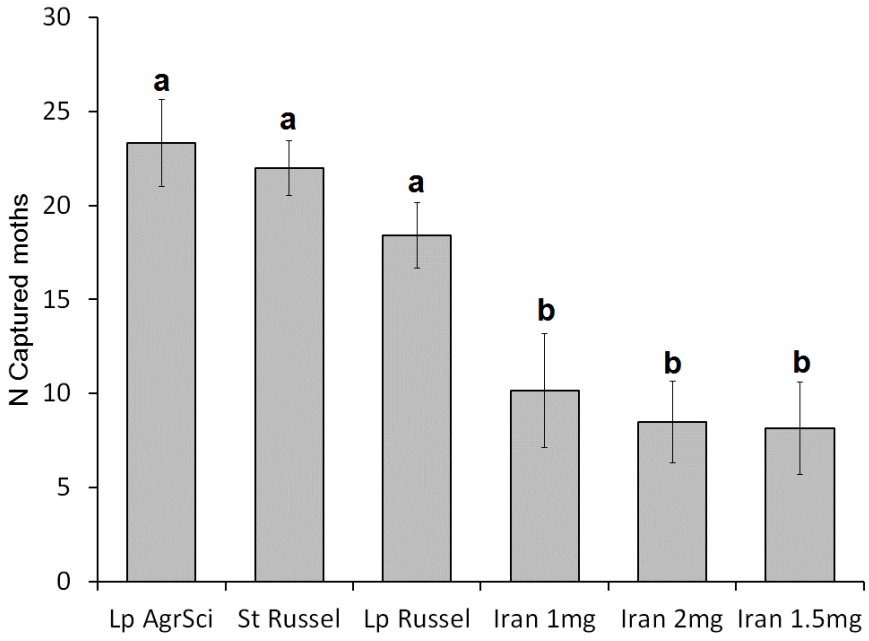


**Fig. 3** the efficiency of six commercially available sexual pheromones in capturing adult leopard moths in Iran.

Totally, the leopard moth sexual pheromone formulated by Agriscience Corporation showed the most efficiency in attracting and capturing of adult males of the leopard moth (an average number of 23.33 adult males were captured by traps containing this pheromone in all studied areas) followed by *S. tipuliformis* (22) and leopard moth (18.41) sexual pheromones formulated by Russel Corporation. However, the efficiency of these three synthetic pheromones was not statistically different (Fig. 3). Iranian formulated pheromones with doses of 1 and 1.5 mg per trap showed the lowest attractiveness for adult males (8.1 and 8.5, respectively) (Fig. 3). Altogether, Iranian pheromones showed a significantly lower efficiency in capturing of the adult leopard moths (t-test: $t=7.72$, $P<0.01$).

## 4 Discussion

We found a significant difference between the efficiency of sexual pheromones formulated by different corporations in attraction and capturing of adult leopard moths in Alborz province and Rabor region. However, there was no significant difference between the efficiency of these compounds in Darremorid and Baft regions (see Fig. 2). The appearance of adult moths was observed to start at mid-May and continue by late August in all studied regions of Kerman province (Baft, Rabor, and Darremorid). An emergence peak was recorded for populations of leopard moth in these three regions from late June to early July. In Alborz province, however, the appearance of adult moths started at mid-June continued to late September with a peak emergence was recorded at middle July. These differences in emergence period and emergence peak may directly be attributable to the differences in climatic conditions of the studied areas especially temperature. The average temperature of the two months, May and June, were 17.35 ºC and 22.7 ºC in Alborz province and 19.7 ºC and 24.4 ºC in the three studied areas of Kerman province. The sexual pheromone of *Z. pyrina* formulated by AgriScience Corporation seems to be the most appropriate compound for mass capturing of adult males in Alborz province and Rabor region (Fig. 3). Although, it showed also a high efficiency in attraction of adult

moths in Baft region somewhat less than the sexual pheromone of *S. tipuliformis* formulated by Russel Corporation (Fig. 3). In Darremorid region, however, it was among the less attractive compounds for the leopard moth. The sexual pheromone of leopard moth formulated by Russel Corporation was always among the most three attractive compounds in all studied areas. We observed a low efficiency in attraction efficiency of the Iranian-formulated sexual pheromones in all used doses (Fig. 3).

Isolated populations are expected to suffer from different environmental selective pressures and experience different random mutations during their long evolutionary history (Vereecken et al., 2007). In this evolutionary route, all biological traits of each population may be evolved different from other populations, a phenomenon that have been proposed to well contribute to genetic divergence and geographic speciation across animal taxa (Panhuis et al., 2001; Vereecken et al., 2007; Symonds et al., 2009). The two major mechanisms proposed to drive allopatric divergence in mating pheromones are stochastic processes (Fisher, 1930; Lande, 1981) and adaptation to local environments (Boughman 2002). These evolutionary forces are not mutually exclusive and might even act in concert, thereby promoting adaptive population divergence over time (Schluter, 2000).

In our experiment, different populations of the leopard moth showed different levels of attraction to the six pheromone kinds we used. The leopard moth sexual pheromone of AgriScience Corporation was the most attractive compound for both populations of Alborz province and Rabor region. Interestingly, we have shown in another study that these two populations are also genetically more related to each other compared to the populations of Baft and Darremorid regions. These similarities may be justified by the fact that Alborz province is the main distributer of walnut sapling in Iran and the population of leopard moth present in Rabor region may be introduced to this region through importation of infected saplings from Alborz province.

Our results imply that, on one hand, the efficiency of sexual pheromones formulated by different companies are not the same and on the other hands, different populations may differ in their response to sexual pheromones. This information well helps us to select appropriate pheromone for exploration, mating disruption, and mass capturing of the leopard moth in each region. Further studies can focus on the attractiveness of different pheromones for populations of the leopard moth adapted to feed on different host trees such as apple. Awareness of these differences may provide some interesting information about the ongoing divergence and potential speciation of these populations.

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