J. Crop Prot. 2019, 8 (2): 215-222

Research Article

Sex pheromone traps for detection of *Cydalima perspectalis* (Walker) (Lepidoptera: Crambidae) in Hyrcanian forests, Iran

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Abstract: The efficacy of sex pheromone traps for detection of *Cydalima perspectalis* (Walker) (Lepidoptera Crambidae) was assessed in 2017 in Cheshmeh-Bolbol Box Reservoir (Golestan province, Iran). Monitoring was done from May to September and three flight peaks were determined. No significant difference was observed between colors as well as heights of installing pheromone Traps for capturing *C. perspectalis*. The results confirmed the efficiency of pheromone traps in decreasing damages of Box tree moth. Also, best time for chemical and pheromone control was assessed.

Keywords: *Cydalima perspectalis*, Pheromone trap, Box tree, Golestan, Iran

Introduction

The box tree, *Buxus hyrcana* (Pojark.), is one of the major and endemic evergreen trees in northern forests of Iran (Caspian Hyrcanian forests). This valuable tree gets infected by twig blight disease of boxwood that causes severe defoliation of boxwood trees (Sabeti, 1995; Mirabolfathy *et al*., 2013).

The next huge shock to *Buxus* trees is created by serious damage of the box tree moth (BTM), *Cydalima perspectalis* (Lepidoptera: Crambidae), which has recently been reported in northern Iran (Ahangaran, 2016; Farahani *et al*., 2016). This moth was introduced into Germany and has spread throughout Europe, causing massive damage to box trees (Krüger, 2008; Leuthardt, 2015). *Cydalima perspectalis* attacks different species of box tree (*Boxus* spp.) and also it is known to damage *Euonymus japonicas* Thunb., *Euonymus alatus* (Thunb.) Siebold (Celastraceae), *Ilex purpurea* Hassk. (Aquifoliaceae), and *Murraya paniculata* (L.) Jack (Rutaceae) (Straten and Muus, 2010). There is no report of the moth infesting other plants except boxes in Europe and Iran (Bella, 2013; Santi *et al*., 2015).

Pheromone traps are the most common tools for detecting insects and decreasing damages of various pests (Howse, 1998). The most common type of pheromone traps is Delta sticky trap (Cardé, 1984). In Italy, Santi *et al*. (2015) used pheromone traps in a park covering an area of 60 ha for detecting *Cydalima prespectalis*. Leuthardt *et al*. (2010) also used pheromone traps for determination of life cycle of box tree moth. Kim and Park (2013) used funnel pheromone traps for detection of box tree moth in Korea.

The aim of this study is evaluation of pheromone traps for detection of seasonal flight activity, determination of the best time for

Handling Editor: Yaghoub Fathipour

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Received: 18 August 2018, Accepted: 21 February 2019
Published online: 22 April 2019

[DOR: 20.1001.1.22519041.2019.8.2.5.7]

control and the effect of pheromone traps on the population fluctuations of *Cydalima prespectalis*, in northern parts of Iran.

Materials and Methods

Studied Area

This study was carried out in Cheshmeh-Bolbol Box Reservoir during 2017 (15 March-30 September) (Fig. 1). This protected area is about 450 hectares and located at 15km southwest of Bandar Gaz city and 10 km south-east of Galugah city and within the range of Cheshmeh-Bulbul and Livan villages, with a longitude of 53° 53′ to 20° 54′ E and a latitude of 45° 36′ to 47° 36′ N (Fig. 1).

This reservoir is mostly covered by *Buxus hyrcana* (Pojark.), and to a lesser extent with *Parrotia persica* Mey, *Quercus castaneifolia* Mey, *Carpinus betulus* Linnaeus, *Acer pseudoplatanus* Linnaeus, *Pterocarya fraxinifolia* (Lam.), *Zelkova carpinifolia* (Pall.) *Mespilus germanica* Linnaeus, *Crataegus rhipidophylla* Gand, *Prunus cerasifera* Ehrh.

About two-third of Cheshmeh-Bolbol reservoir is located on a steep slope that is not accessible by man.

Preparing pheromone traps

160 standard sticky Delta traps were suspended from trees at about 1.2-2m above ground, using 20-gauge galvanized steel wire and at an average distance of 80-100m between pheromone traps in different locations of the reservoir (in about 24ha, 6-7 traps/ha) (Fig. 2). Pheromone traps were provided by Econex® Company (Spain). Traps were baited with a pheromone vial impregnated with 2mg Z11-hexadecenal + E11-hexadecenal (with 98% min purity). Captured males BTM were counted and recorded every other day. Traps were replaced as they become damaged or dirty, whereas pheromone lures were replaced every 4-5 weeks.

Experiment 1 Determination of the Optimum Color of Pheromone Traps for Capturing *Cydalima perspectalis*

68 green color and 92 white color traps were used for this experiment (Fig. 3). The traps were distributed randomly between boxwood trees at the approximate distance of 80-100m betweentraps. Captured males of BTM were counted and recorded every two days till the end of moth's last generation activity.

Figure 1 Map of Cheshmeh-Bolbol Box Reservoir (Golestan province) where this study was done.

[DOR: 20.1001.1.22519041.2019.8.2.5.7]

Figure 2 Map of pheromone traps (blue dots) located in Cheshmeh-Bolbol Box Reservoir, Golestan province.

Figures 3 Two different colors (white and green) of delta shape pheromone traps installed in Cheshmeh-Bolbol for capturing *Cydalima prespectalis*.

Experiment 2 Determination of the Optimum height of Pheromone Traps for Capturing *Cydalima perspectalis*

Traps were fixed at 3 different heights above the ground: 1-1.30m (44 traps), 1.30-1.60m (63 traps) and 1.60-2m (53 traps). Captured males of BTM were counted and recorded every two days till the end of the moth's last generation activity.

Experiment 3 Survey on detection of BTM with the aim to find the right time for chemical and pheromone control

In order to detection of BTM, number of adult male moths captured in traps were counted every two days during its activity form 15 March-30 September 2017. The average temperature and average precipitation in the area ranged from 4.3 to > 27 °C and 0-72mm, respectively.

[DOR: 20.1001.1.22519041.2019.8.2.5.7]

Experiment 4 Surveying the efficiency of pheromone traps in decreasing damages of BTM

For evaluation of Delta traps' efficiency in decreasing damages of BTM, about 6-7 traps were installed per hectare based on the technical data of pheromone usage. The number of adult male moths captured in each trap were counted every two days.

Analysis of Data and Drawing Chart

Statistical analysis of data was carried out using SPSS statistics v22 (IBM Corp, 2013) and drawing chart was done using Microsoft Excel ver. 2010 (Microsoft Office Professional Plus 2010, Version 14.0.7015.1000).

Results

The use of pheromone traps seems to be alternative method in controlling and monitoring of various pests. In this study, using of colored pheromone traps against *Cydalima prespectalis*, a new pest of Box in Iran, exhibited promising results.

Experiment 1

Statistical analysis, confirmed that there was no significant difference between mean number of captured moths by green and white color pheromone traps at the 95% confidence interval, P-value > 0.05 (α = 0.149, T value = 1.44, df = 151) (Table 1).

Table 1 Statistical analysis (T-test) of 160 pheromone traps for colors of traps.

Color of traps	Number of traps	Mean captured moths	Standard deviation	T	Significant level
Green	68	42.89	17.82	1.44	0.149
White	92	38.40	16.14		

Experiment 2

Analyzing the variance between three different heights of trap installation showed that there was no significant difference in the number of captured *Cydalima perspectalis* among the height of pheromone traps installed at the 95% confidence interval (α = 0.087, P-value > 0/05) (Table 2).

Table 2. Analysis of variance (ANOVA) between 3 different installation heights of traps.

Height of traps above ground	Number of traps	Captured moths	Standard deviation	F	Significant level
1.0 - 1.3 m	44	45.85	20.83	2.48	0.087
1.3 - 1.6 m	63	36.81	16.98		
1.6 – 2.0 m	53	36.36	13.27		

Experiment 3

The results showed that there are three periods of adult emergence for BTM per year in Golestan province (Fig. 4). The first flight of male moths originated from the overwintering third generation larvae of the previous year. Our continuous observations indicated that overwintering was carried out by the 3rd and 4th instar larvae of the third generation protected between 2-3 leaves in a light silk cocoon. In late winter (8-10 March) larvae began activity, becoming mature and pupating on 15-20 April. The adult moths of the overwintering generation emerged from early May to late May, when first catches occurred (first flight, 9 May); the second one from early July to late July, the third from Mid-August to Mid-September. The rate of captures in pheromone traps was increased and reached a peak in all generations. The population peak of male adults for the 1st, 2nd and 3rd generations occurred in 16-May, 24-July and 14-August, respectively.

These different results can be explained by the weather conditions, as in Mid-August-September, the temperature was high and precipitation low (Fig. 5), that's why the decreased abundance of BTM and severe interference and irregularity in 3rd generations.

Experiment 4

Delta shape traps caught totally 8204 BTM males during 2017; 162 males in first flight; 6500 males during the second flight and 1542 males in third flight. Large numbers of male moths were captured in the second generation,

[DOR: 20.1001.1.22519041.2019.8.2.5.7]

resulting in an imbalance in the sex ratio, which impacts the mating pattern of *C. prespectalis*, and as a result in the last generation infertile eggs were encountered (Fig. 6).

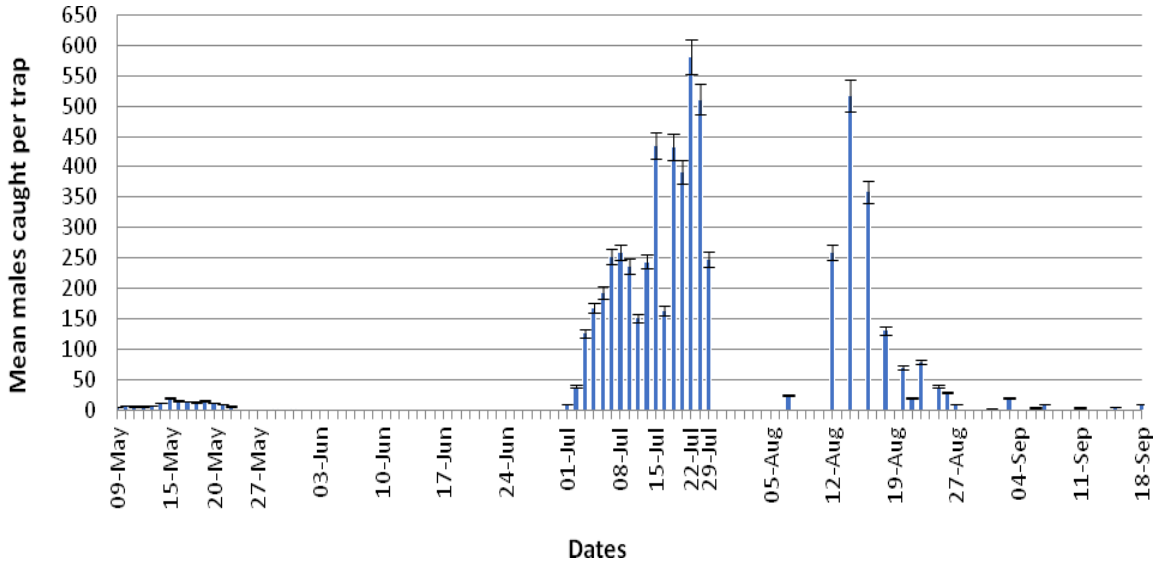

Figure 4 Population fluctuation (mean ± SE) of *Cydalima perspectalis* males caught per trap in 2017 in "Cheshmeh-Bolbol box reservoir, Golestan province".

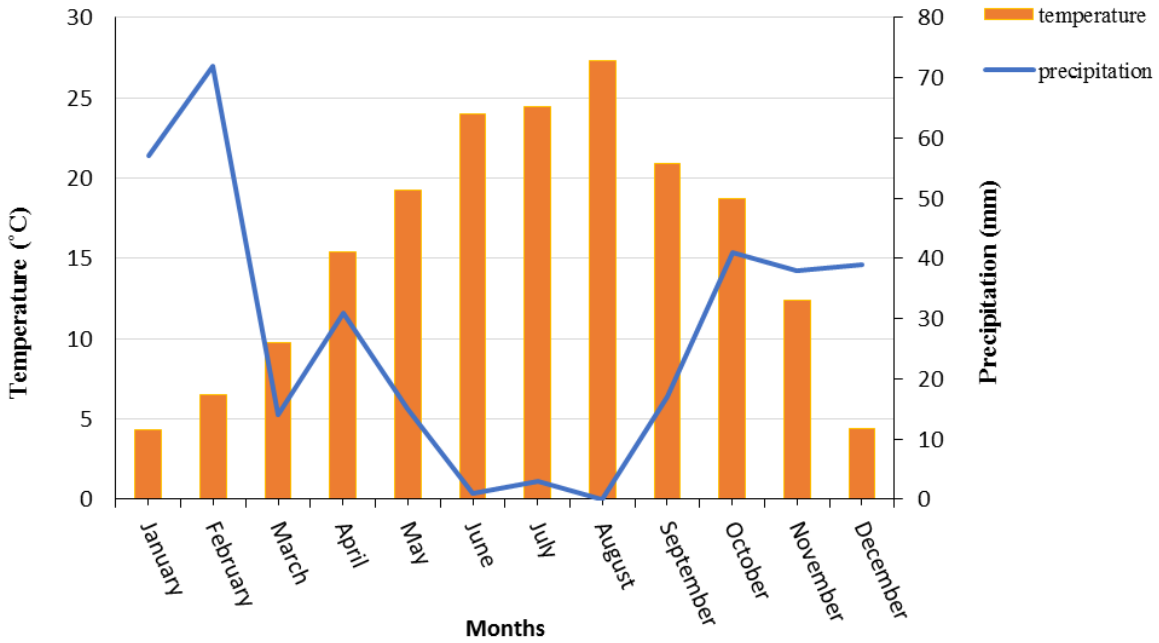

Figure 5 The mean monthly temperature (°C) and precipitation (mm) in Cheshmeh-Bolbol box reservoir, Golestan province.

[DOR: 20.1001.1.22519041.2019.8.2.5.7]

Figure 6 Infertile eggs of *Cydalima prespectalis* from 3rd generation.

Discussion

The results presented in this paper match with the results of sex pheromone trapping data in Europe, as Leuthardt *et al*. (2010) and Santi *et al*. (2015) reported three generations in Switzerland and Italy. But some researches show that *C. prespectalis* has two generations in northwest Switzerland (Leuthardt and Ramin, 2011; Nacambo *et al*., 2014). These differences could be due to various degree-days needed per generation (Nacambo *et al*., 2014). On the other hand northern Switzerland has relatively lower temperature than the south that can be an effective factor on the needed time for larval development as well as number of BTM generations.

Our finding indicated that the population of *C. prespectalis* was low at the onset of the season. The population trended to increase as the season advanced then started to decrease later in the season. Different results were reported by Santi *et al*. (2015), as an increasing trend of the number of moths was observed toward third flight.

Based on the results the best time for chemical control is concurrent with emergence of neonate larvae (1-10 days after emergence of adults), for the first generation is between 22 May-5 June, in 2nd generation is between 12 July-3 August and in 3rd generation we are faced with overlapping generation but it can be between 17 August-12 September. Also the best times for installing pheromone traps for the three generations are 1 May-27 May (1st generation), 27 June-2 August (2nd generation) and 7 August-27 Sep (3rd generation).

Infertile eggs resulted by sex ratio disruption in last generation of BTM in the studied area that showed obvious decrease in moth population. Richerson *et al*. (1976) stated that unmated females often start depositing unfertilized eggs and even if mated they have a reduced ability to deposit fertile eggs.

Kawazu *et al*. (2007) and Santi *et al*. (2015) stated that the baited traps with only 2 sex pheromone instead of three components are efficient, this study confirms their results too.

Conclusion

The results confirmed that the delta traps baited with sex pheromone are very efficient in capturing BTM males in order to estimate flight trends and plan for its control.

Although chemical insecticides have been used to control this pest in various parts of the world, they cause environmental and human health concerns that cannot be ignored. Thus the use of pheromone is expected to reduce application of chemical pesticides in pests control programs.

Acknowledgements

We would like to thank Dr. Ebrahim Sharifi Ashourabadi (Research Institute of Forests and Rangelands, Tehran, Iran) for his kind help in data analyzing and two anonymous reviewers for their valuable comments on the earlier version of this paper.

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[DOR: 20.1001.1.22519041.2019.8.2.5.7]

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[DOR: 20.1001.1.22519041.2019.8.2.5.7]

ردیابی شب‌پره شمشاد *Cydalima perspectalis* (Walker) (Lepidoptera Crambidae) توسط تله‌های فرمون جنسی در جنگل‌های هیرکانی، ایران

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چکیده: کارایی تله‌های فرومون جنسی شب‌پره شمشاد *Cydalima perspectalis* (Walker) (Lepidoptera Crambidae) در سال ۲۰۱۷ در ذخیره‌گاه شمشاد چشمه بلبل (استان گلستان) مورد بررسی قرار گرفت. ردیابی آفت از فروردین تا مهر ماه انجام شد و سه اوج پرواز برای این شب‌پره تعیین شد. اختلاف معنی‌داری بین رنگ و ارتفاع نصب تله‌های فرومونی در به‌دام انداختن *C. perspectalis* مشاهده نشد. نتایج به‌دست آمده از این پژوهش بیانگر کارایی تله‌های فرومونی در کاهش خسارت شب‌پره شمشاد است. هم‌چنین در این پژوهش بهترین زمان برای کنترل شیمیایی و استفاده از فرومون مورد ارزیابی قرار گرفت.

واژگان کلیدی: *Cydalima perspectalis*، تله فرومونی، شمشاد، گلستان، ایران

[DOR: 20.1001.1.22519041.2019.8.2.5.7]