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Flight patterns of the European corn borer, *Ostrinia nubilalis*, in Slovenian hop gardens in 1999-2016

Magda Rak Cizej[1], **Pasquale Trematerra**[2]
[1]*Plant Protection Department, Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia*
[2]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy*

Abstract

The suitability of a light trap (from 1999 to 2016), and pheromone traps and pheromone + phenylacetaldehyde (PAA) baited traps (from 2010 to 2012), in the monitoring of European corn borer (ECB), *Ostrinia nubilalis* (Hubner), moth flights in Slovenian hop gardens was studied. In 18 years light trap collected a total of 13381 specimens (with an average of 743 specimens per year), the number of ECB varied from a minimum of 184 specimens in 2014 to a maximum of 2060 specimens collected during 2003. From 2010 to 2016, of the 5327 specimens captured, 3778 were males (70.70%) and 1566 were females (29.30%). In general, during the ECB first annual fly the captured moths increased after the third week of May, with a peak on 10 June; between the last week of June and the second week of August, the ECB second fly can be observed with a peak during the last week of July; third annual fly was revealed from last week of August to last week of September with a peak of adults at the end of August. Pheromone and pheromone + PAA traps (wing sticky and mesh netting cone models) demonstrated low suitability for capturing ECB males and females. Adult trapped by light trap and pheromone and pheromone + PAA traps, respectively, displayed similar pattern. Average daily temperature and precipitation level influenced the flight patterns of ECB moths. ECB field infestations in hop gardens could be surveyed by counting egg masses on plants and observing signs of early damages by first-instar larvae. Monitoring of moth flights by means of easily handled pheromone traps provides valuable information.

Key words: European corn borer, *Ostrinia nubilalis*, monitoring, *Humulus lupulus*, light trap, pheromone traps, pheromone + phenylacetaldehyde traps.

Introduction

Some 2600 farms in the European Union (EU) grow hops (*Humulus lupulus* L.), covering 30391 hectares in 2016, accounting for 54.3% of the total surface area used for hop-growing worldwide (Hopsteiner Newsletter, 2016). Hops are grown in 14 EU countries. Some 18600 hectares are used for hop cultivation in Germany; the other main EU producers are the Czech Republic, Slovenia, Poland, and the UK. The EU is one of the hubs of the global market in hops. With regard to external trade, the EU has traditionally been a net exporter. Over the last few years, the surplus has amounted to some 20000 tonnes of cone equivalents. The main buyer is Russia, followed by the United States and Japan.

Production of high quality hops requires careful attention to numerous arthropod pests and diseases. The damage of these organisms may cause ranges from insignificant to complete loss due to direct reduction in quantity of yield or diminished yield quality that can render hops not marketable. In Europe, among others, the larvae of several moths and butterflies attack hop; however, only the European corn borer (ECB), *Ostrinia nubilalis* (Hubner) (Lepidoptera Crambidae), commonly reaches damaging levels (Janus and Stengel, 1973).

In Slovenia, ECB has been known as a maize and hop pest for a long time and has recently also caused significant economic damage to vegetable crops and ornamental plants (Rak Cizej *et al.*, 2012). It is a serious pest of hop in most parts of Savinja valley, causing yield losses and affecting the quality of the hop. In the last 10 years, the ECB presence increased significantly and was also observed to increase economic damage to host crops, especially hop and maize (Rak Cizej *et al.*, 2012).

In the centre of Slovenia, where there are many hop gardens, ECB has two generations per year on hop and maize. The larvae of the first generation cause the most damage by boring into the hop stems in May and early of June, while in the second generation (in July) larvae bore into leaf and hop cones. ECB larvae are responsible for stunted growth and the development of plants, which results in a substantial decrease in yield quality and quantity. Hop plants damaged by ECB larvae are completely destroyed in dry and hot seasons (Rak Cizej *et al.*, 2009; 2012). For management of the ECB population, in Slovenia the use of a contact insecticide is recommended, with a.i. lambda-cyhalothrin limited to 2 times a year in the same area or a.i. *Bacillus thuringiensis* subsp. *kurstaki* limited to 3 times per year (Rak Cizej *et al.*, 2012).

In the central part of Slovenia, the E-strain of ECB is present on hop and maize. In addition to the two known pheromone strains (Z and E) (Anglade *et al.*, 1984), there are three known ecotypes of ECB, which differ in voltinism (univoltine, bivoltine and multi-voltine ecotypes). In some areas, more than one ecotype and sex pheromone strains occur sympatrically (Showers, 1993; Rak Cizej *et al.*, 2010). ECB female fecundity is strongly related to strains and ecotypes; females that have mated multiple times are significantly more fecund than females that mate only once. The eggs are usually laid on the lower leaf surfaces or directly on fruit. In a healthy population, 95% of fertile eggs hatch. The sex ratio is 1:1 and a slight proterandry has been detected by some authors (i.e. males emerge hours or days earlier than the females) (Fadamiro and Baker, 1999).

ECB moths are active at night; females release a sex pheromone on the night of emergence or on the 2-3 subsequent nights of adult life (Camerini *et al.*, 2015).

ECB field infestations in hop gardens can be surveyed by counting egg masses on plants and observing signs of early damages by first-instar larvae (but these operations are hard to realize because plants of hop are high). Monitoring of moth flights provides valuable information on the timing of oviposition.

At this regard in the present study, we analysed the suitability of light trap (from 1999 to 2016), and pheromone traps and pheromone + phenylacetaldehyde (PAA) traps (from 2010 to 2012), for the monitoring of pattern of ECB moth flights in Slovenian hop gardens.

Hop is a perennial plant, with an average growth in hop gardens of around 15 years. In the spring time, at the end of March every year, plants were cut to make them grow very quickly; in May, an average daily growth of 15-20 cm is reported in good weather conditions (high temperature and rainfall). At the end of May, plants are high 6.5 m or more, in June the plants form lateral shoots, in July they start flowering and in August the fruit develops (in cones). In the start of September, it is time for harvesting, depending on the cultivars.

Materials and methods

Light trap

The experiment was conducted from 1999-2016 (for 18 years), in a hop garden on a farm located in Žalec, Slovenia (46°14'N 15°09'E, 256 m a.s.l.). For ECB moths monitoring a light trap was installed in the hop garden with access to the power grid. The light trap had a 160W mercury bulb Osram-Hwl and an inbuilt weekly programmable digital time (XBS KO-2, Poland). The trap was located in the hop garden at a distance of 16 m from the experimental field's edge. The trap was 1 m high, with a plastic container (with a bottom tank containing chloroform 30-50 ml/night), covered with a fine net attached to the edge of the lamp to collect attracted insects. The trap transmits light around 350 and 400 nm, with most being at 362 nm (Rak Cizej *et al.*, 2014). The light trap was switched on every day starting from the third week of April (from 20.00 to 06.00), and the ECB moths collections was carried out until third week of September. The number of adults trapped was checked daily. The examination of the specimens collected was performed in the laboratory of Slovenian Institute of Hop Research and Brewing in Žalec. From 2010 to 2016, ECB moth specimens were also separated by sex.

Pheromone traps

Trapping of ECB moths was performed for 3 years from the end of May until the third week of September (2010-2012), in parallel with light trap. In 2010 wing sticky trap (Traptest, Isagro, Milano, Italy) and in 2011-2012 mesh netting cone traps (CORETRAP, Riff98, Bologna, Italy). The sticky traps were baited with rubber septa loading 0.1 mg of (97:3) E:Z-11-tetradecenyl acetate (Isagro, Milano, Italy). The mesh netting cone traps were baited with pheromones + (PAA) (Isagro, Milano, Italy) for catching both sexes of ECB moths.

All the pheromone traps were placed on two locations in hops: one location was 100 m away from the light trap in Žalec (location 1) (46°14'N 15°09'E, 256 m a.s.l.), while the second trap was placed in hops at Roje near Žalec (location 2) (46°14'N 15°08'E, 260 m a.s.l.). All pheromone traps were located on the edge of the hop garden, at a height of 1.2 m from the ground.

Pheromone rubber septa were changed every 14 days, and PAA attractants were changed every 30 days. Moths caught by traps were counted every week. The examination of the material collected was performed in the laboratory.

Meteorological data

At Žalec, near a hop garden, 10 m from a light trap, an AdconTelemetry GmbH meteorological stage (Klosterneuburg, Austria), was positioned to record temperature, precipitation and relative humidity data every 15 minutes (figure 1 and supplemental figure S1).

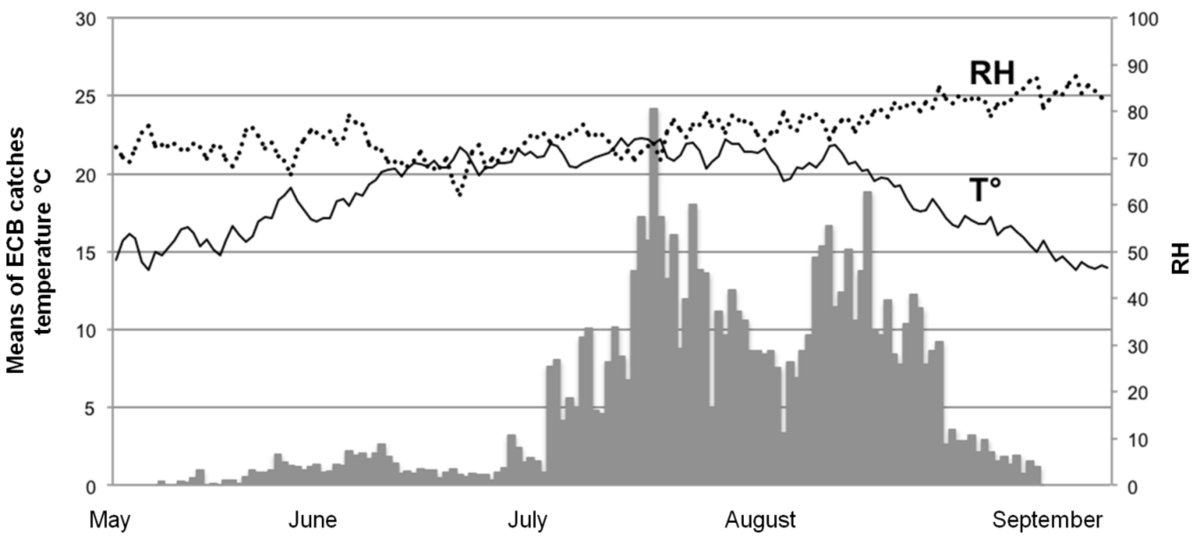

Figure 1. Flight ECB moths patterns revealed by the light trap in hop garden, average data 1999-2016.

Results

ECB flight patterns based on light trap catches

As reported the ECB moths adult flights were monitored for 18 years at the Žalec location (figure 1 and supplemental figure S1). Males and females were collected by light trap, amounting to a total of 13381 specimens (with an average of 743 specimens per year). From 2010 to 2016, of the 5327 specimens captured, 3762 were males (70.62%) and 1565 were females (29.38%). From 1999 to 2016, the number of ECB moths trapped in light trap varied from a minimum of 184 specimens in 2014 to a maximum of 2060 specimens collected in 2003.

During those years, the first moths were captured in the first week of May, when the hop was 3.5-4 m high; captures were realized until the end of September, which was the end of the hop harvesting time (figures 1 and 5).

In the first ECB flight the number of captured moths substantially increased after the third week of May, with a peak on 10 June when plants were in the end of bine growth stage. Between the last week of June and the second week of August, was observed the ECB second fly, with a peak during the last week of July. The third annual fly was observed from the last week of August to the last week of September, with a peak of adults on the last week of August (figure 1).

In the entire period (1999-2016), 299 adults were trapped in May, 665 in June, 5190 in July, 5972 in August and 1255 in September.

From 1999 to 2016, major weather parameters varied significantly. The temperature changed during the moth flights: between June and September, the average air temperature varied from 12.0 to 21.8 °C. Rainfall occurred from June to mid-September; it was most intense in the first week of July (40.0 mm), the second week of August (51.1 mm) and the first week of September (115.6 mm) (figure 1 and supplemental figure S1).

In the analysed 18-year period, the most favourable weather conditions for ECB moth flights were recorded in 2003; in that year, 2060 moths were captured in the light trap. On the contrary, weather conditions in 2014 were less favourable for ECB moth flights; in that year, 184 moths were captured in the light trap (figure 2). Numbers of ECB moths seems different between the years. In 2003 and 2015 there were similar higher catches (figure 2).

From 2010 to 2016 a total of 5344 adults were captured with light trap, of which 3778 males (70.70%) and 1566 females (29.30%) (figure 3).

ECB flight patterns based on pheromone traps and pheromone + PAA trap catches

We had the first successful attempts with pheromone + PAA traps in 2011, where were used mesh netting cone traps at locations 1 and 2. The trap at location 1 (Žalec), which was 100 m away from the light trap, captured 28 males and 1 female; at location 2 (Roje near Žalec), throughout the season, 130 males and 10 females were caught (figures 4). In location 1, the mesh netting cone traps baited with pheromones + PAA captured the first 3 males of ECB on 29 May. The maximum number of males was recorded on 19 July (6 specimens) and on 16 August (6 specimens), while the last individuals were recorded on 21 August (4 specimens). The only female was trapped on 9 August (figure 4). In location 2, the mesh netting cone traps collected the first 12 males on 29 May. The maximum number of males was recorded on 12 July (23 specimens) and 31 July (15 specimens), while the last individuals were recorded on 21 August (9 specimens). The females were trapped on 4 July (1 specimen), the maximum number was recorded on 12 July (6 specimens), while the last individuals were recorded on 16 August (2 specimens) (figure 4).

In 2010, we attempted to catch ECB with pheromone wing sticky traps, but this was unsuccessful. Same negative trapping was observed in 2012 using the mesh netting cone trap baited with pheromones + PAA.

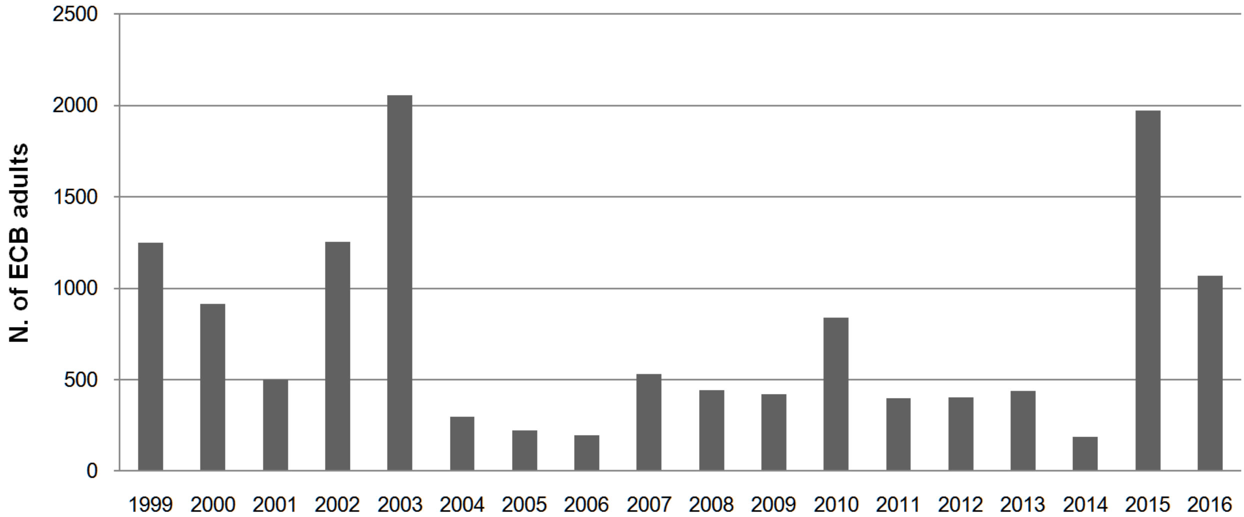

Figure 2. Yearly catches of ECB revealed by light trap in hop garden, data from 1999 to 2016.

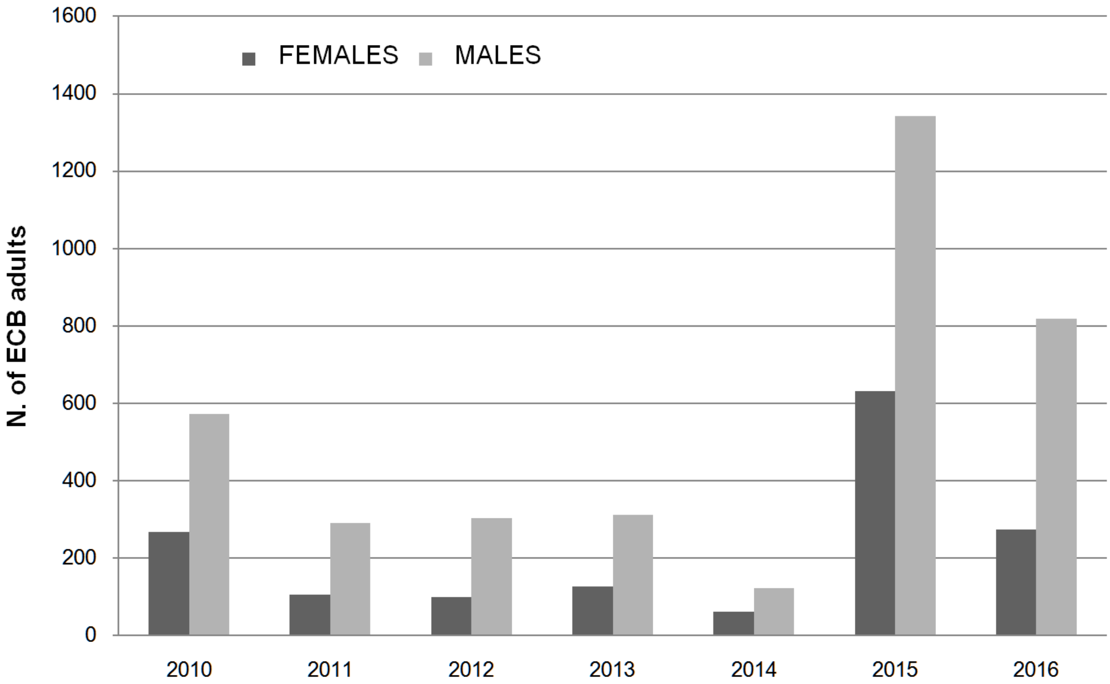

Figure 3. Females and males of ECB caught by light trap in hop garden from 2010 to 2016.

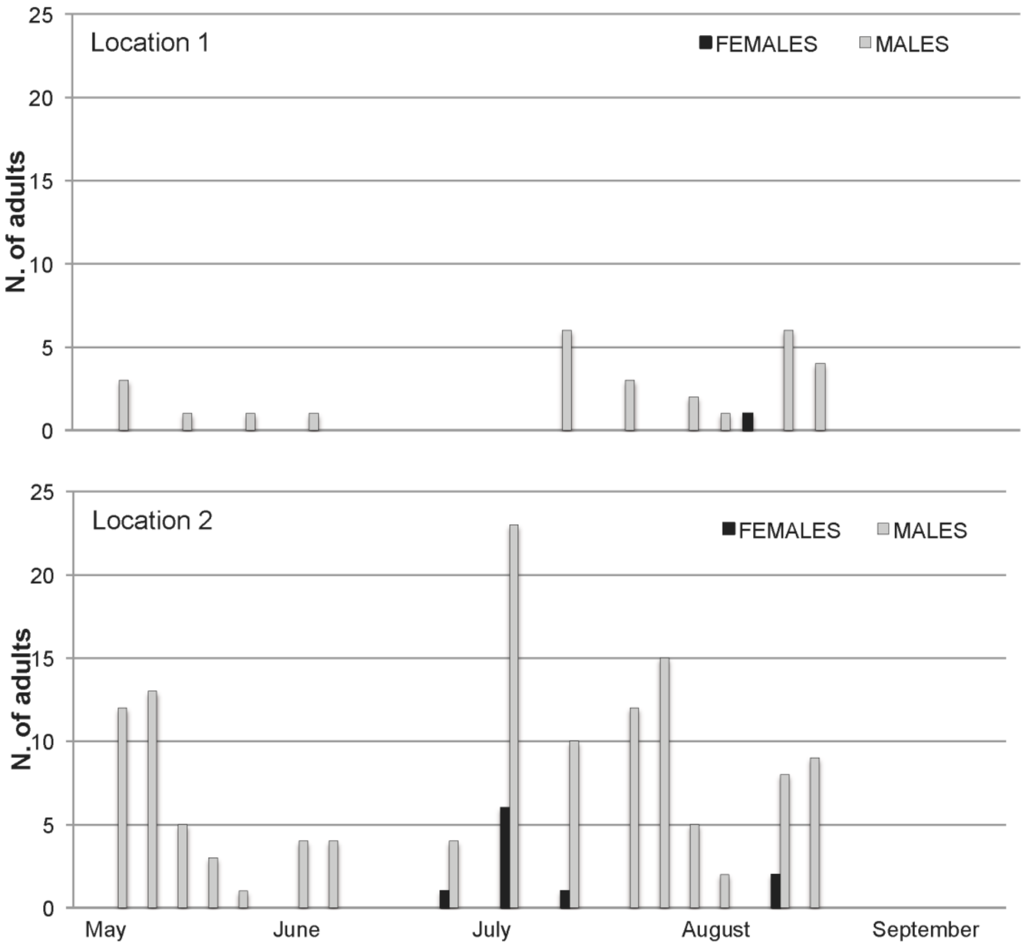

Figure 4. Females and males of ECB caught by mesh netting cone trap baited with pheromone + PAA in 2011 (locations 1 and 2).

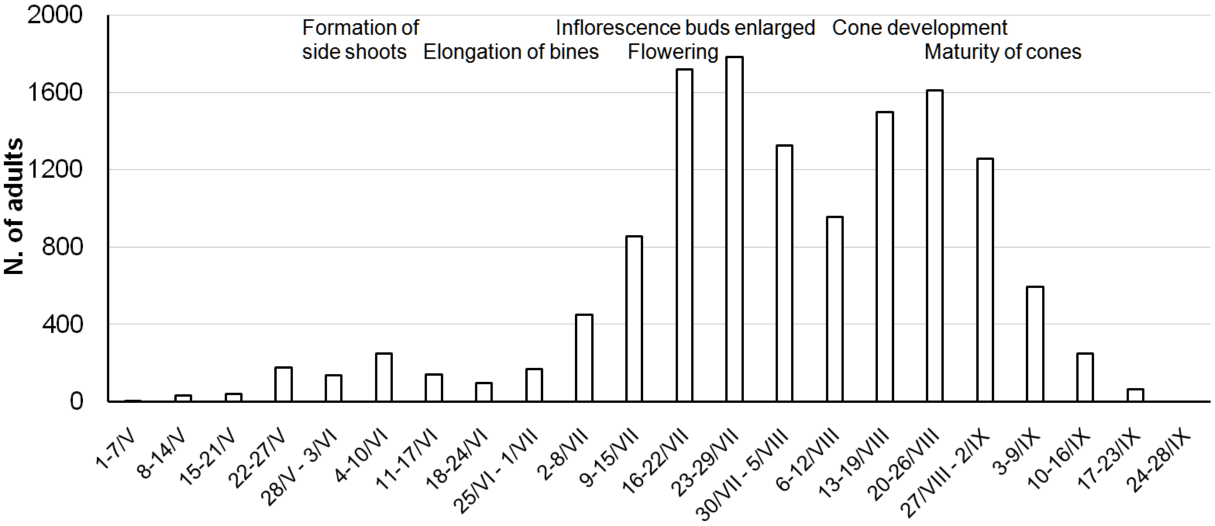

Figure 5. ECB moths caught by light traps in hop gardens from 1999 to 2016 (average data) in relation to phenology of hop stages.

Discussion

Most of the references regarding the ECB economic impact is relative to damage on maize. There are reports of damage to other crops of economic value, but details of the total crop impact are lacking. The extent of damage caused by ECB varies from year to year and between geographic locations within years. Some of this variation is due to the number of generations in a geographic location, climatic conditions for a given year, physiological stage of the crop at infestation, and natural mortality (Mason *et al*., 1996; Bohn *et al*., 1999; Keszthelyi and Lengyel, 2003).

The identification of the optimal timing for the ECB control in hop gardens is a prerequisite for its effectiveness (figure 5). In fact ECB larvae are exposed to natural enemies or to insecticides only for a short period before they start to bore into the plants. Because the optimal time for biological and chemical methods to ECB control is limited, the monitoring is of key importance for the identification of optimal timing for treatments. A practical method still used for identification of the treatment date to ECB control is based on the hop growth stages. The treatments date should be carried out during almost all the hop development i.e. not feasible. This method, however, is biased with a large error, because factors such as the date on which hop plants started growing, or the effect of weather conditions on pest and crop growth are not taken into account. Therefore, other methods need to be used which are based on observations of the ECB individual developmental stages on hop gardens. For such purposes, in maize, light traps and pheromone traps for capturing ECB moths are used, or samplings of ECB egg masses (Beres, 2012).

Due to presence of different ECB strains, the monitoring using sex pheromones sticky traps was not always adopted in IPM. As it is known there are two distinct ECB strains, which differ in the ratio of Z to E isomers of 11-tetradecenyl acetate. Hybrid males from crosses between the two strains produce a pheromone blend with an isomeric ratio of 65E:35Z (Klun and Maini, 1979) but ECB males generally respond to a broad range of isomeric ratios (Glover *et al*., 1987; Linn *et al*., 1999; Pélozuelo *et al*., 2004). Sympatric populations of the E and Z strains as well as inter-strain hybrids exist at some locations (Klun *et al*., 1975; Anglade *et al*., 1984; Roelofs *et al*., 1985; Peña *et al*., 1988; Showers, 1993).

When sex pheromone traps are used for monitoring moth flights, it is critical that the pheromone blend used to bait the traps is representative of the blend being used by the local natural ECB population (Sorenson *et al*., 1992; Bourguet *et al*., 1999). Although moth catches in pheromone traps frequently fail to provide a quantitative indicator of the larval infestation that subsequently develops, monitoring of moth flights provides valuable information on the oviposition timing.

If we compare data of captures from 2010-2012, made by the light trap and the pheromone traps, at Žalec (location 1) is possible to note big differences in number of specimens caught. In 2010, the light trap caught a total of 831 ECB moths (571 males). In the following two years 2011 and 2012, the total moth caught were less (277 and 294 males respectively). In parallel with light trap, ECB males caught with pheromone traps were unsuccessful in 2010 and 2012. In 2011 only we caught 28 ECB males and 1 ECB female at Žalec (location 1), while at location 2, 130 males and 10 females were caught.

The lack of success in ECB monitoring with sticky pheromone traps could be due to the low amount of glue applied on the bottom. The mesh netting cone traps capture approximately six times more ECB males than sticky traps. Furthermore the low number of collected moths is probably related to the faraway distance of the traps from ECB action sites (Showers *et al*., 1976; DeRozari *et al*., 1977; Mason *et al*., 1997).

It remains unclear whether flight curves plotted from pheromone trap captures truly reflect moth phenology. This point has been repeatedly raised (Bartels and Hutchinson, 1998; Pélozuelo and Frérot, 2006), but no study has been specifically designed to address the question (Pélozuelo and Frérot, 2007). In the ECB control, both trap design and trap placement are important determinants of trap efficiency and the possibility of combining the sex pheromone with another lure to attract also females has been demonstrated (Maini and Burgio, 1991; 1994; 1999; Burgio and Maini, 1995; Mason *et al*., 1997; Bartels and Hutchison, 1998; Camerini *et al*., 2015; Karpati *et al*., 2016).

Our data indicated the low precision of pheromone traps in monitoring of the ECB flight pattern under hop garden conditions, anyway could be that pheromone traps are easily handled than light traps.

The number of moths captured by pheromone traps appeared lower than the number of moths captured by the light trap, but in the monitoring the number of the catches is not relevant. Similar results were also reported by Żołnierz and Hurej (2007) and by Beres (2012).

The light trap is one of the most reliable methods of monitoring moths, and the Slovenian Institute of Hop Research and Brewing in Žalec has used it for more than 35 years to monitor ECB on hops (Rak Cizej *et al*., 2009). The downside of a light trap is that it is not practical, it cannot be used for ECB monitoring at all locations, and, furthermore, it is an expensive method.

As reported, in last 10 years, the ECB population has increased substantially in Slovenia hop gardens (Rak Cizej *et al*., 2012). The reasons could be attributed to different factors which primarily include: untimely removal of leftovers of host plants after the harvest, the application of contact insecticides in agriculture having been restricted, phytosanitary and hygienic measures not being implemented rigorously enough, climate change, etc.. Nonetheless, sanitation by ploughing at the end of the host-plant growing season could provide interesting results (Rak Cizej *et al*., 2012).

In Slovenia, for the ECB larvae control in hop gardens, spraying contact insecticide has proven to be insufficiently effective (Rak Cizej *et al*., 2012). On the contrary, control using formulations of *Bacillus thuringiensis* subsp. *kurstaki* generally gives good results, reducing the ECB population to 65%, particularly when the treatments are correctly applied and target the first larval stage (Rak Cizej *et al*., 2012). Early planting could reduce the exposure of the crop to ECB damage, but this agronomic practice cannot be adopted in hop growing because it is a perennial plant.

According to our results, the indications by pheromone traps and light trap, respectively, displayed a similar pattern (figures 4 and 5).

The average daily temperature and the weekly precipitation level had a potential effect on the flight patterns of ECB moths. The number of rainy days in July was relatively low in comparison to other study months, which, in combination with high temperatures, facilitated moth flights. We observed that the duration of ECB moth flight monitored with the light trap is correlated with changes in weather conditions. In 2003, when weather conditions were favourable for the flight, the ECB was present in the second week of August. However, in the rainy years 2001, 2005 and 2014, less ECB moths were caught and the flight ended late, at the end of August (supplemental figure S1). A similar correlation was found by Kania (1961) and by Beres (2012), who reported that ECB flight pattern was the longest in relatively cold periods with low precipitation. The flight was slightly shorter in warm and rainy periods, and shortest during hot and drought weather.

In our studies, the first moths found in the light trap were usually females, but the number of males remained higher until the end of pest occurrence on the hop garden. Males dominated among the ECB captured, this data was not confirmed by other authors like Kania (1961) and Żoł nierz and Hurej (2007), who reported females to be dominant. According to Mason *et al*. (1997) the different results derive mainly from the placement of the trap in the field.

In our case, the light trap recorded the maximum number of ECB moths in the first and in the second weeks of July and August. In the pheromone traps, the maximum number of ECB males were recorded in the first and second week of July.

The light and pheromone traps data together with samplings of ECB eggs masses offer more credible information of ECB population development and subsequent sustainable control methods.

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Authors' addresses: Magda RAK CIZEJ (corresponding author, e-mail: magda.rak-cizej@ihps.si), Plant Protection, Department, Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, 3210 Žalec, Slovenia; Pasquale TREMATERRA, Department of Agricultural, Environmental and Food Sciences, University of Molise, via De Sanctis, 86100, Campobasso, Italy.

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