

## Pheromone-based monitoring of the European corn borer (*Ostrinia nubilalis*) in Hungary

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### Abstract

Despite the fact that the pheromone structure and composition of the European corn borer (*Ostrinia nubilalis*) was identified in the early 1970s, an effective pheromone-based monitoring method of this species has not been established yet in Hungary. The aim of this study was to find an optimal monitoring strategy for this economically important pest using pheromone traps. We compared three trap designs, five ratios and four doses of the earlier identified pheromone components in three different locations in Hungary. In the first year there was no significant difference between the delta and the cone traps as both were able to catch males in sufficient numbers. However, in the second year the cone traps captured significantly more males. In a comparison of the different ratios of the two pheromone components, our results demonstrate that the usage of 97:3 Z:E [97% (Z)11-tetradecenyl acetate and 3% (E)11-tetradecenyl acetate] ratio attracted the highest number of males. Therefore, we suggest that the Z pheromone strain exists in the three monitored regions. In the experiment, where the different doses were compared, there was no significant difference in the number of males caught. In 2015, the flight dynamics of the species showed that males start to fly in the beginning of June and the highest flight peak occurs in mid June. Based on our results we conclude that in Hungary the pheromone traps are able to attract and monitor European corn borer males using the appropriate trap design, dose and ratio under field conditions.

**Keywords:** *Ostrinia nubilalis*, European corn borer, pheromone, trap design, monitoring

### Introduction

The most important aim of integrated pest management (IPM) is to spatiotemporally predict the existence of pests to determine efficient insecticide delivery timing (Lefebvre et al, 2014). One of the most effective way to monitor pest insects in the field is with pheromone traps, which can help in the estimation of the time and the number of target insects in the selected field and can also suggest the appropriate time for insecticide delivery when the number of target species reaches the maximum. The pheromone traps are species specific; therefore, can only catch the target species as they only contain the synthetic sex-pheromone of the species to which all of the males belong. In comparison, blacklight traps catch all nocturnal insects, including endangered ones and other non-target species. The biweekly checking of the traps can help estimate the population dynamics; climax of expected egg laying and consequently the emergence of larvae can be predicted as well. In view of these results it is possible to decide on the necessity and synchronized timing of plant protection treatment (Baker, 2008).

The European corn borer (ECB, *Ostrinia nubilalis*, Hübner, Lepidoptera: Pyralidae) can cause, specifically under traditional extensive cultivation conditions, the greatest damage worldwide, which is approximately €1.6 billion/year (FAOSTAT, 2016). The ECB is also one of the most serious pests in Hungary in terms of the country's one million hectares of harvested maize (Szőke et al, 2002). This polyphagous species is native to Europe, living on more than 200 host plants (Ponsard et al, 2004). Despite its polyphagous lifestyle, this species primarily prefers maize (*Zea mays* L) since its introduction to Europe. The newly hatched larvae initially chew on the abaxial side of the leaf, and then tunnel into the maize stalk; at this time it is very difficult to eliminate them. Larvae are tunnel in the stalks, which become soft and weak, which even a strong wind can break it down. Furthermore, at the point where the larvae enter into the stalk, various species of molds can be colonised; therefore, these infested stalks can be dangerous for livestock (Logrieco et al, 2003). The damage from the second generation can be more serious since the emerged larvae could also attack the ear, wherein the

micotoxin producing *Fusarium* molds could be colonized, which are harmful also for humans (Blandino et al, 2015).

Due to the pheromone polymorphism of the species, two pheromone strains exist. The Z-strain females produce 97% (Z)-tetradecenyl acetate and 3% (E)-tetradecenyl acetate, whereas the E-strain females produce the opposite ratio of these two pheromone components (Klun and Robinson, 1971; Klun et al, 1973; Anglade et al, 1984), which has been extensively studied in terms of both pheromone production and preference (Kárpáti et al, 2007; Kárpáti et al, 2010; Lassance et al, 2010; Kárpáti et al, 2013; Koutroumpa et al, 2014). Despite the fact that the ratio of the two pheromone components are very different, in sympatry the strains can interbreed and the ratio of the hybrids could reach even 15% (Klun and Huettel, 1988; Dopman et al, 2010).

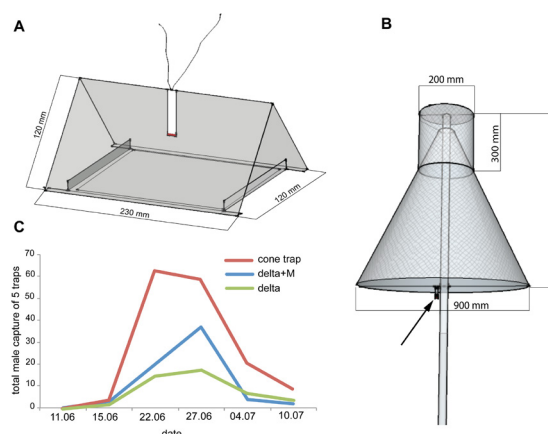
Notwithstanding the fact that the pheromone composition and the existence of the two strains were determined in the early 1970s, problems related to the monitoring of the species with pheromone traps have still not yet been solved in Hungary. In fact, Maini and Burgio (1999) demonstrated that in Italy the pheromone traps are not consistently attracting ECB males. However, based on the previous studies it is obvious that the reliable monitoring strategies of this species strongly depend on the position and the design of the trap (Pelozuelo and Frerot, 2006). Earlier studies revealed that only the accurate mixture of the two pheromone components can attract males in the selected field: the absence of the minor component in the mixture can block the attractiveness of the pheromone trap (Klun et al, 1973; Carde et al, 1975; Kalinova et al, 1994). Moreover, the amount of the pheromone in the dispenser can also affect the trap's ability to capture the males (McLeod and Starratt, 1978; Kalinova et al, 1994). Therefore our main aim was to focus on the developing of a reliable and reproducible monitoring system which is consistently able to monitor the ECB in Hungary using different trap designs, ratios and doses.

## Materials and Methods

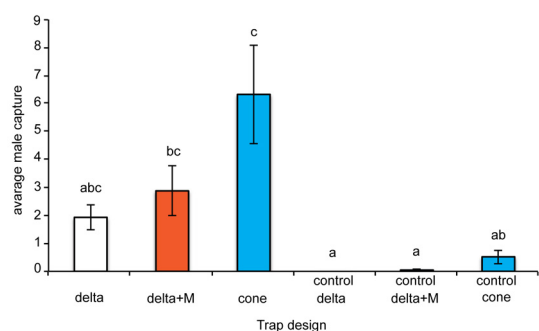
The trapping experiments in 2013 and 2014 were carried out in Törökszentmiklós, Jász-Nagykun-Szolnok County, Hungary (47°05'59"N; 20°28'20"E). In 2015, the experiments were expanded and performed in two other locations - Bicske (47°28'34"N; 18°36'28"E) and Martonvásár (47°19'57"N; 18°49'21"E), Fejér County, Hungary. At all the locations the traps were placed at the edge of a maize field. For the experiments, the delta traps were provided by Csalomon® (Budapest, Hungary; Figure 1A) and the cone traps were home-made according to Hartstack et al (1979; Figure 1B). The cone traps were hanged on the top of a pole in the edge of the maize field. The delta traps were hanged in the first row and on the highest point of the maize plant

since, based on the literature, it is known that the effectiveness of the pheromone traps are highest if the traps are placed on the highest point of the canopy (Mason et al, 1997). Throughout the experimental period all delta traps were elevated to the highest point due to the growth of the maize plant.

Based on the previous visual inspection at the experimental field regarding the ECB population density, the traps were placed in Törökszentmiklós on 11 June 2013 and on 6 June 2014; in Bicske on 5 May 2015; and in Martonvásár on 2 June 2015, respectively. In all the experiments the traps were equipped with Wheaton rubber septa (Wheaton, Miliville, NJ, USA) and 20 µl of the different below-mentioned concentrations were applied. The two pheromone components, (Z)- and (E) tetradecenyl acetate, were purchased from Pherobank (Wijk bij Duurstede, The Netherlands). In 2013, three different experiments were carried out. The first experiment compared the effectiveness of three different trap designs: a) Delta trap with original sticky sheet (delta); b) Delta trap with mouse glue sticky sheet (Biotoll-Unichem, Vrhnika, Slovenia; active ingredient: polibutene (delta+M)); c) cone trap (cone) and the corresponding unbaited controls. In this experiment we used a 97:3 Z:E ratio of the two pheromone components and a 10 µg dose. In the second experiment the different ratios of the two pheromone components were compared. The delta+M trap design and 5 different ratios of the two pheromone components: Z:E 99:1; 97:3; 93:7; 50:50; 1:99 were tested. In the third experiment different doses of the pheromone blend were compared; delta+M trap design, 97:3 Z:E ratio and four different doses: 0.1, 1, 10, and 100 µg were tested. In 2014 we compared three different trap designs such as delta, delta+M and cone traps baited with 10 µg at a 97:3 Z:E pheromone ratio. In 2015, at two locations, we used a delta+M trap design and compared three different ratios of the two pheromone components



**Figure 1** - Schematic illustrations of delta (A) and cone (B) trap design used for attracting ECB males. Arrow indicates the position of the pheromone lure. (C) Comparison of captured ECB males in delta, delta+M, and cone traps during one-month period in 2013.

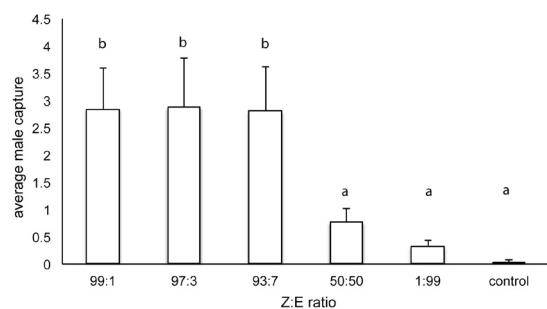


**Figure 2** - The average of ECB male capture with different pheromone trap designs ( $n = 5$ ). The traps were placed in Törökszentmiklós in 2013. The total load of the pheromone on the dispensers were  $10 \mu\text{g}$  in a ratio of 97:3 Z:E. delta – delta shaped trap with original sticky sheet; delta+M – delta shaped trap with mouse glue sticky sheets; cone – cone shaped trap. Different letters indicate significant differences ( $p = 0.05$ ).

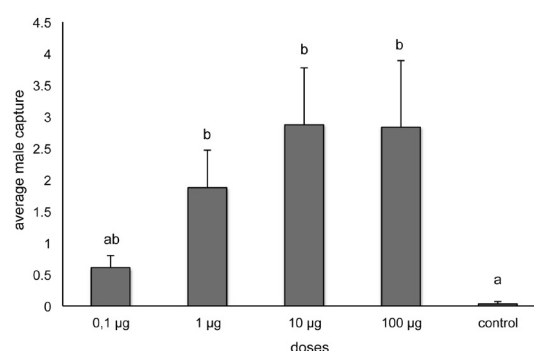
(97:3, 50:50, 1:99 Z:E); the loaded dose was  $10 \mu\text{g}$ . In all places and years, the pheromone lures in the traps were replaced every three weeks, the control traps did not contain pheromone lures. The experiments were carried out in five replicates, the distance between the traps was 20m and the traps were randomly placed within a replicate. The traps were checked and the sticky sheets were replaced each week. Data collected during the three experimental years, were analysed with SPSS 17 (IBM Corporation, Armonk NY, US) statistical software. Data were normalised using  $\log(x+1)$  transformation and ANOVA statistical model was used. If the ANOVA showed significant differences, then Tukey's posthoc test was used to separate treatment means (Day and Quinn, 1989). In all statistical tests the significance level ( $p$ ) was 5%.

## Results

In all three experiments in 2013, a total of 655 males were captured. In the first experiment we compared three different trap designs regarding their capacity to capture males. The total capture for all the traps was 354 males during the monitoring period. All



**Figure 3** - The average of ECB male capture with different ratios of the two pheromone components in Törökszentmiklós in 2013 ( $n = 5$ ). Delta+M traps and  $10 \mu\text{g}$  dose were used. Different letters indicate significant differences ( $p = 0.05$ ).

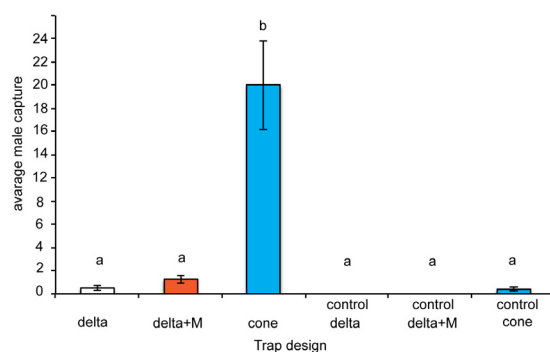


**Figure 4** - The average of ECB male capture with different doses loaded in the pheromone lures in Törökszentmiklós in 2013 ( $n = 5$ ). Delta+M traps and a 97:3 Z:E ratio of the two pheromone components were used. Different letters indicate significant differences ( $p = 0.05$ ).

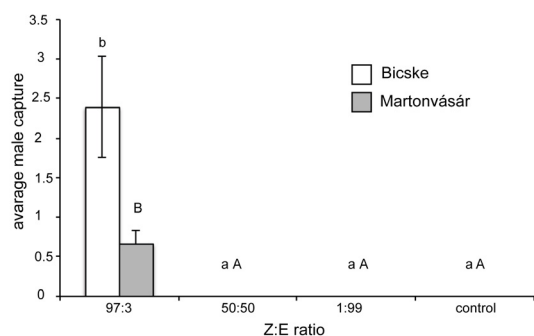
the different trap designs captured significantly more males compared to the corresponding unbaited control traps. The cone trap captured the most males (in total 158), although it did not differ significantly from other trap designs' capture (Figure 2). All trap designs captured the males at the same time; however the cone trap has a higher catching capacity due to the larger size of the trap (Figure 1C).

In the second experiment we compared the attractiveness of the different ratios of the two pheromone components. The traps baited with 99:1, 97:3 and 93:7 Z:E ratios captured the highest number of males compared to the other ratios (50:50, 1:99) which results did not differ significantly. The 50:50 and 1:99 Z:E ratios did not differ from that of the control capturing (Figure 3).

In the third experiment the different doses of the pheromone amount loaded onto the dispenser were compared. When onto the dispenser 1, 10, and 100  $\mu\text{g}$  was applied, the traps captured significantly high-



**Figure 5** - The average of ECB male capture with different pheromone trap designs ( $n = 5$ ). The traps were placed in Törökszentmiklós in 2014. The total load of the pheromone on the dispensers were  $10 \mu\text{g}$  in a ratio of 97:3 Z:E. Delta – delta shaped trap with original sticky sheet; delta+M – delta shaped trap with mouse glue sticky sheets; cone – cone shaped trap. Different letters indicate significant differences ( $p = 0.05$ ).



**Figure 6** - The average of ECB male captures with different ratios of the two pheromone components ( $n = 5$ ). The traps were placed at Bicske and Martonvásár, Fejér County, Hungary, in 2015. The total load of the pheromone on the dispensers was  $10 \mu\text{g}$ . Delta traps with mouse glue sticky sheets were used. Different types of letters (uppercase and lowercase) are used for comparison of different places. Different letters indicate significant differences ( $p = 0.05$ ).

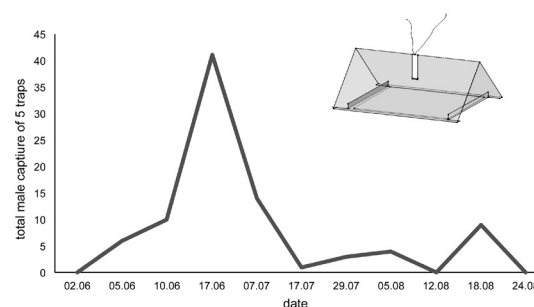
er number of males compared to the unbaited ones. The lowest dose ( $0.1 \mu\text{g}$ ) attracted the lowest number of males but this number did not significantly differ from 1, 10, and  $100 \mu\text{g}$  doses and not even from the control traps (Figure 4).

In 2014, where three different trap designs were compared, we captured a total of 201 ECB males. There were no significant differences between the delta, delta+M trap designs and the corresponding controls (control delta, control delta+M); however, the cone traps captured a significantly higher number of males compared to the delta and delta+M traps. The capturing efficacy of the cone traps significantly differs from that of the control cone traps (Figure 5).

In 2015, in two other places in Hungary, three different ratios of the binary mixture were tested to clarify which strain of the species exists. The results show that only the delta+M traps, containing a 97:3 Z:E ratio of the two pheromone components, captured males (Figure 6). Based on the capture of the pheromone traps in Bicske during the summer, the flight dynamics of the species were also outlined (Figure 7). The flight of the ECB started in the beginning of June (2 June) and lasted until the end of August. The traps captured the highest number of males in the middle of June (17 June).

## Discussion

In this study, we investigated the development of a reliable, sex pheromone-based monitoring strategy against ECB in Hungary. We compared three different trap designs, five different ratios of the earlier identified pheromone components and four doses. Based on the behavior of the ECB, we know that the trap design is a crucial factor in catching males (Pelozuelo and Frerot, 2006; Laurent and Frerot, 2007) and, therefore, we tested different traps in order to find the most suitable one for monitoring. In 2013,



**Figure 7** - Flight dynamics of the European corn borer based with delta+M pheromone trap captures in 2015, Bicske, Hungary. Delta+M traps baited with  $10 \mu\text{g}$  of 97:3 Z:E pheromone binary mixture were used.

the cone traps did not catch significantly more males (Figure 2); however, in 2014 at the same place these traps attracted a significantly higher numbers (Figure 5). In comparison, the cone trap attracted the highest number of males in other countries also (Webster et al, 1986; Maini and Burgio, 1990; Bartels and Hutchison, 1998; Pelozuelo and Frerot, 2006). This discrepancy could be due to the different population density between the two years. Based on the results of our trap design experiment, we conclude that both delta and cone traps are able to sufficiently monitor the ECB population. In previous studies, where the delta-shaped traps were placed in a dense grassy area surrounding the maize field, only a small number of males were attracted to the traps (Webster et al, 1986; Stewart, 1994; Pelozuelo et al, 2006; Pelozuelo and Frerot, 2006). In those studies it was hypothesised that the grassy area can be an aggregation site for males and females (Showers et al, 1976; Derozari et al, 1977). In contrast, in our study we placed the delta traps as high as possible on the maize stem. Therefore, this could be the reason more males flew into our delta traps in 2013. Laurent and Frerot (2007) also reported that the height of the delta traps plays an important role in capturing the male ECB. Earlier observations also showed that the wire mesh cone trap's vertical position is important in terms of the efficiency of the traps (Derrick et al, 1992; Mason et al, 1997; Ngollo et al, 2000). From an economic and practical perspective, the delta trap is more feasible to use compared to the cone trap because it is less expensive and more convenient to place the trap and then replace the sticky sheets. Therefore, in the second and the third experiment in 2013, we used the delta trap design.

In 2013, in the second series of experiment we compared the different ratios of the two pheromone components to determine which strain exists in that certain area (Figure 3). Based on our results the trap baited with 97:3 Z:E and similar ratios (93:7 and 99:1 Z:E) captured the most males; therefore, we conclude that in this field the Z pheromone strain of the species exists. These results correspond to earlier findings



where [Pena et al \(1988\)](#) also found Z-strain based on pheromone gland extraction analysis, approximately 90 km from our experimental field. In our previous study, based on the female pheromone gland extract analysis, we also found Z blend composition in the south of Hungary ([Kárpáti et al, 2007](#)).

In the third set of experiments in 2013, we compared the attractiveness of different doses of the 97:3 Z:E binary pheromone blend ([Figure 4](#)). The 1 µg dose lost its attractiveness earlier due to suboptimal loading. Most males flew into the traps, which were baited with 10 and 100 µg doses, although tested doses did not show significant differences. However, the traps loaded with a 10 µg dose captured males earliest in the flight season, while those with a 100 µg dose started to catch males two weeks after the experiment started (data not shown). We assume that this is due to the fact that the amount of the pheromone in the 100 µg dose dropped to a significantly lower level, the optimal dose to attract males, over the two-week period. We found contrary results in former literature: [McLeod and Starratt \(1978\)](#) showed that 100 µg is the most efficient amount, while, [Kalinova et al \(1994\)](#) found that 10 µg is the optimal dose to attract males.

In 2015, we investigated which strain of the ECB exists in the northwest part of the country; therefore, we compared three different ratios of the binary mixture using delta+M traps in Bicske and Martonvásár. We found that in both places the delta traps loaded with the Z-strain's pheromone composition attracted males only ([Figure 6](#)). In Martonvásár, the traps captured fewer males compared to the site at Bicske, which could be due to earlier insecticide treatment. Based on these results we conclude that in both southeast and northwest part of Hungary the Z-strain exists and, therefore, the Z pheromone lures are effective in attracting ECB. In Bicske, we were also able to monitor the flight dynamics of the species ([Figure 7](#)). Based on the results we found that the traps captured the highest number of males in mid June. Earlier studies show that the first flight peak occurs at the end of June and the beginning of July in Hungary based on the adult captures of light traps ([Keszthelyi, 2006](#); [Keszthelyi et al, 2006](#)). In the north of Italy, the first flight climax appears one week earlier, at the end of May, according to pheromone trap captures, which findings commensurate with ours ([Camerini et al, 2015](#)).

Based on our results we conclude that we were able to find a suitable trap design, with the appropriate ratio and pheromone dose, which can be successfully used to monitor the ECB population in Hungary. This pheromone-based monitoring system, besides predicting the population density of the target species, can also help in determining the timing of Trichogramma-based oophagus parasitoid treatments and insecticide application. Future studies can focus on examining the effect of trap height in dense grassy area compare to maize field, and also investi-

gate the attractiveness of appropriate host plant volatiles in combination with sex pheromones. Moreover, our findings may help to contribute to the application of mating disruption using a suitable pheromone blend.

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