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Efficiency of pheromone baited traps for monitoring of the European corn borer *Ostrinia nubilalis* (Lep.: Crambidae) in Mazandaran province

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ABSTRACT

Pheromone baited sticky traps are used as a monitoring tool for the survey of the European corn borer (ECB) populations in Iran. However, they fail to provide any valuable information to agricultural authorities. This is possibly due either to a wrong formulation of the pheromone lure for local moth populations or to the sticky trap design itself. This trap design is generally considered as poorly efficient against the ECB moth. In this paper, we (1) investigate the pheromone type of Iranian ECB females by means of gas-chromatography and gas-chromatography coupled to mass spectrometry and (2) we compare the efficiency of the delta sticky-trap *versus* a home-made wire mesh cone trap in a field test. Pheromone analyses of 14 individual females clearly showed that their pheromone composition is similar to the pheromone of the ECB *Z* strain feeding on corn in France, i.e. *Z*11-14:OAc as major component and *E*11-14:OAc and 14:OAc as minor components present on the gland surface. This trapping experiment showed that home-made wire mesh cone traps are more efficient

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than sticky traps (respectively 258 males caught *versus* 1) and confirmed that a pheromone lure releasing a *Z* type pheromone is an efficient attractant for ECB males from the Iranian population feeding on corn.

Key words: European Corn Borer, pest monitoring, pheromone baited trap, wire mesh cone trap, sticky trap.

Introduction

The European Corn borer, *Ostrinia nubilalis* Hübner (1796), is a major pest of corn in Iran where the moth population is controlled either by means of a massive release of the oophagous parasitoid wasp *Trichogramma* sp. or by an application of insecticide. However, both the biological agent and the insecticide molecules target a specific instar of the pest. *Trichogramma* females kill ECB eggs -and only eggs- by parasiting them, while insecticides mainly kill neonate larvae as older larvae gets inaccessible to the insecticide by boring into the host plant stem. As a result, the treatment efficiency is dependant on the right timing between the treatment application date and the ECB developmental stage. An accurate adult-ECB flight survey is therefore necessary to ensure the efficiency of the control strategy. Sex pheromone baited traps are good candidates to play such a role. The sex pheromone emitted by ECB females to attract the males has been identified in the United States and Europe as a blend of *Z* and *E*-11-tetradecenyl acetate, *Z*11-14:OAc and *E*11-14:OAc. Within the species, two pheromone strains have been described: a "*Z* strain" (also called the "*Z* race") in which females release and males respond to a blend with 97% *Z*11-14:OAc and 3% *E*11-14:OAc (Klun *et al.*, 1973; Kochansky *et al.*, 1975) and an "*E* strain" that uses the opposite blend made of 96% to 99% *E*11-14:OAc and 4% to 1% *Z*11-14:OAc (Klun *et al.*, 1973; Carde & Roelofs, 1978). Iranian agriculture authorities have planed field trapping of ECB males with both blends but the capture levels were too low to describe accurately the flight curve of adults ECB and to be able to estimate the flight peak date (A. Espahbodi, unpublished data). Moreover, Asgary (1994) assumes that the *Ostrinia* species which is present in Iran belongs to an *Ostrinia nubilalis* sub-species referred to as *Ostrinia nubilalis persica*. This brings up the possibility that ECB population in Iran could have a different pheromone composition and thus it could explain why males are not caught with classical *Z* or *E* pheromones lures in this area.

In order to understand the difficulties experienced previously with ECB field trapping in Iran, we analysed the pheromone of individual ECB females by means of gas chromatography and gas chromatography coupled to mass spectrometry. We also compared male captures in

delta sticky traps *versus* captures in wire mesh cone traps (WMC-traps). Webster *et al.* (1986), Maini & Burgio (1990) and Bartels & Huchison (1998) already showed that wire mesh cone traps are more efficient than sticky traps in the United States and Europe. Based on Pelozuelo (2004) description that only about 6% of attracted males are caught in delta sticky trap design, we tested the hypothesis that low captures of ECB males could rather be due to trap design than to low efficiency of the pheromone lure to attract male insects.

Material and methods

Female sex pheromone identification:

1- Insects: ECB were collected as 5th instar larvae in corn stalk in the Mazandaran province (Sari region). Larvae were sent to the "Phytopharmacie et Médiateurs chimiques" research unit in France and completed their life cycle on a corn-based artificial diet (Poitout & Bues, 1970). The sexes were separated as pupae. Newly emerged females were kept individually in plastic containers supplied with water under rearing room conditions: 16:8h L:D, T =24°C ± 2, R. H.= 60 % ± 10. Two to five day-old females were used for sex pheromone collection within the last three hours of scotophase, during their calling period.

2- Pheromone collection: Pheromones were collected by SPME as described by Frérot *et al.* (1997). The pheromone gland was extruded by a gentle pressure on the abdomen and kept in this position with metallic forceps. A Supelco SPME fibre (65 µm Carbowax^{TM}-Divinylbenzene), previously cleaned by thermal desorption (5 min in the GC-injector at 240°C), was gently rubbed on the pheromone gland for 4 min at room temperature. Care was taken to avoid contact with scales and anal droplets. Then each fibre was either directly analysed or wrapped in an aluminium foil and stored at –20°C until analysis.

3- Pheromone analysis: The chemical components of the sex pheromone were identified via gas chromatography (GC) for seven females and gas chromatography coupled to mass spectrometry (GC-MS) for 10 females.

GC procedure: A Varian 3400 gas chromatograph equipped with a split-splitless injector and a polar column RtX Wax (RESTEK, 30 m, 0.32 mm ID, 0.5 µm df) was used. The components adsorbed onto the SPME fibre were subjected to thermal desorption for 2 min. in the injector heated to 250°C. The column temperature was programmed to increase from 50°C to 100°C at a rate of 15°C min^{-1} then to 245°C at 5°C min^{-1}; helium at a pressure of 11 psi was the carrier gas.

GC-MS procedure: A Varian 3400 gas chromatograph equipped with a SPI injector and coupled to a Saturn II mass spectrometer (ion trap type) was used. The column was an

apolar column MDN-S (SUPELCO, 30 m, 0.32 mm ID, 0.25 µm df). Both polar and apolar column can be used for the successful identification of the *Z* and *E* tetradecenyl isomers. Temperature conditions were as follows: injector temperature 250°C, initial column temperature 50°C during 1 min, then temperature increased to 300°C at a 8°C min^{-1} rate. Desorbtion duration in the injector was 2 min and helium at a pressure of 11 psi was the carrier gas.

Compounds were identified by comparison of retention times (gas chromatography) and mass spectra (mass spectrometry) of the natural compounds with those of synthetic reference samples. In order to allow an easy comparison between pheromone blends whatever their number of components, the ratio between the different components are given as (*Z*:*E*)/*X* with *Z*=percentage of *Z*11-14:OAc related to the total quantity of *Z* plus *E*11-14:OAc, *E*=percentage of *E*11-14:OAc related to the total quantity of *Z* plus *E*11-14:OAc, *X*= ratio of the considered component related to a total amount of *Z* plus *E*11-14:OAc arbitrary fixed as 100.

Field trapping: Delta sticky traps versus Wire mesh cone traps:

1) Traps description: Delta sticky-traps were provided by NPP Calliope (Noguère, France) and were 28 cm long, 20 cm large and 11 cm high (fig. 1).

Wire mesh cone traps were home-made following the indications of figure 1.

2) Trapping site and trap placement: One delta sticky-trap and one WMC-trap were set up in three sites (i.e. 3 replicates). Traps were placed in a grassy border, close to a corn field and at least 50 m apart from each other. Delta sticky-traps were hung about 40 cm above the ground level and WMC-traps were set with the pheromone caps 10 cm below the grass canopy, as indicated by Mason *et al.* (1997) for optimum ECB capture. The 3 trapping sites were located in Iran, in the Mazandaran province (Sari region). The maximum distance between sites was about 20 km. Trapping took place from July 7th 2003 to August 10th 2003 and traps were checked on an average 4-day basis intervals (max. 7 days, min. 2 days) to count and remove ECB males.

3) Pheromone lures. Pheromone lures were red septa loaded with a synthetic blend containing *Z* and *E*11-14:OAc in the 97:3 ratio, purchased from Biosystem (France). These lures are specifically formulated for attracting *Z* strain. Pheromone lures were replaced once, on July 20th 2003.

Result

Female sex-pheromone identification:

GC analysis: The analysis of pheromone glands of seven ECB females indicated that the major component of the pheromone blend is the *Z*11-14:OAc. This component was detected in all of the seven pheromone collections. Two other products were also present on the gland surface: *E*11-14:OAc and 14:OAc. *E*11-14:OAc was present with no doubt in 2 samples characterised by high quantity of *Z*11-14:OAc and was present as traces in one sample. The saturated compound 14:OAc was present in 6 samples out of 7. Average ratios of pheromone components were 98.2/1.8±0.4 for *Z*11-14:OAc/*E*11-14:OAc ratio (N=2) and 100/16.4±5.4 for (*Z*11-14:OAc +*E*11-14:OAc)/14:OAc ratio (N=6) (Table 1).

GC-MS analysis: Ten female glands were analysed via GC-MS. In seven glands, the *Z*11-14:OAc isomer could be identified as the major component of the pheromone blend. Out of those seven female glands, two glands contained *Z*11-14:OAc as major component and *E*11-14:OAc as minor component. The *Z*11-14:OAc/*E*11-14:OAc ratios of those female were 98.6/1.4 and 99.3/0.7 (Table 1). The compound 14:OAc could not be detected in those seven females containing pheromone products, due probably to the use of an apolar column for GC-MS analysis that does not enable separating *Z*11-14:OAc and 14:OAc.

Field-trapping: Two-hundred and fifty nine males were captured (total for all sites). Total capture achieved in each sites are different: 3.9% of all males were captured in site 1 while 45.9 and 50.2% were caught in sites 2 and 3 respectively. This difference is statistically significant ($Khi^2 = 101.9$, $df = 2$, $\alpha = 0.05$, comparison of observed data *versus* theoretical data calculated under the hypothesis of an equi-distribution of male captures among sites 1, 2 and 3). The temporal variation of the male capture followed the same pattern in site 2 and 3 (figure 3). In both sites, high levels of ECB-male capture were recorded on July 20th and 23rd: 2.8 and 7.7 moths per night in site 2 and 8.8 and 5.7 moths per night in site 3. High capture levels were also recorded at the end of the trapping period on August 10th: 15 moths per night in site 2 and 8 moths per night in site 3.

Out of 259 caught males, 258 were captured in a wire mesh cone trap, i.e. 99.6%. Only one individual was caught in a delta sticky trap. This male was captured within the four days following the trap set up. The difference between capture in delta sticky traps and capture in WMC traps is highly significant ($Khi^2 = 255.1$, $df = 1$, $\alpha = 0.05$, comparison of observed data *versus* theoretical data calculated under the hypothesis of an equi-distribution of male captures among trap types).

Discussion

Female sex-pheromone identification: The pheromone blend extracted from the ECB females of Mazadaran region clearly belongs to the "*Z* blend" type. All female pheromone glands contain *Z*11-14:OAc as major component and the *E*11-14:OAc compound is identified as minor component in a 97 / 1.4 *ratio* on four female glands. Based on this ratio, we calculated *E*11-14:OAc quantities expected for the five other females. In all cases the expected quantity was below the G-C detection threshold. Thus, the *E*11-14:OAc absence is not indicative of a lack of this compound in the pheromone blend initially released by the female. The *Z*11-14:OAc / *E*11-14:OAc ratio evidenced on the two Iranian ECB females is slightly different from the *Z*/*E* ratio usually described as 97 / 3 for *O. nubilalis* (Klun *et al.*, 1973, Kochansky *et al.*, 1975) but the difference could not be statistically tested with 4 individuals. Furthermore, it is noteworthy that the technique of contact-SPME applied with *Z* females from France also indicated a lower proportion of the *E* isomer, i.e. 97.7 / 2.3 ± 6.3 (N=66) (Pélozuelo *et al.*, 2004). The 14:OAc is present on the pheromone gland in a (*Z*11-14:OAc+*E*11-14:OAc) / 14:OAc ratio of (100) / 16.4. As this saturated component is a biosynthesis precursor for both *Z* and *E*11-14:OAc (Glover & Roelofs, 1988), its presence could be expected. 14:OAc is also detected on the pheromone gland of ECB females from France, in a similar ratio of 100 / 17.1 (N=61) (Pélozuelo *et al.*, 2004) and many authors also signal 14:OAc presence when analysing ECB gland wash (Kochansky *et al.*, 1975; Klun & Junk, 1977; Attygal *et al.*, 1987; Strubble *et al.*, 1987; Peña *et al.*, 1988; Kalinova *et al.*, 1994). The implication of 14:OAc for female detection by ECB males can not be rejected. However as far as we know only one field trapping study evidences a synergist effect of 14:OAc when added to the usual *Z* blend (Stockel, 1980).

Field trapping: Results obtained over a one-month trapping period clearly indicate that the pheromone lures loaded with the typical *Z* blend attract Iranian ECB-males. This point is consistent with the analysis of pheromone glands from females coming from the same area. Pooled together, those results show that even if ECB from Iranian populations may belong to the *O. nubilalis persica* sub-species described by Mutuutra & Munroe (1970), no pheromone divergence occurred between "*Z*" ECB-populations from Iran and "*Z*" ECB populations from Europe and USA. The results presented in this paper show that Iranian agriculture authorities are not dealing with a new pheromone strain of ECB but with the usual *Z* strain. The comparison of pheromone baited delta sticky-traps *versus* pheromone baited WMC traps clearly indicates that the trap design is a key factor for a successful ECB population monitoring. Webster *et al.* (1986), Bartel & Huchison (1998), Maini & Burgio (1990) and

Pélozuelo (2004) previously evidenced the superiority of WMC trap over sticky traps. Pélozuelo (2004) showed that males are equally attracted in delta and WMC traps but that a lower proportion of attracted males is captured in delta sticky-traps. Our study also demonstrates that WMC traps are far more efficient than delta sticky traps. WMC traps allow high enough levels of ECB capture to describe accurately the flight patterns of the species. The ECB flight pattern obtained with WMC traps in the present study was characterised by two peaks corresponding to first and second flight of adults ECB and thus could be used to correctly position *Trichogramma* release or insecticide application. However, in site one, ECB capture levels obtained with a WMC trap were low, resulting in a non-informative flight curve. This may be due either to a locally low ECB infestation or to a problem with the trap position.

Conclusion

Taking into account this study and previous comparison of wire mesh cone traps and sticky traps, we recommend the use of WMC traps for ECB management in corn fields in Iran. Although we did not evaluate the trap position effect, we suggest that extension service agents to put the traps in grassy borders known as aggregation sites for ECB moths. The accurate position of the trap might be a key factor for ECB capture. Our results also indicate that the *Z* blended pheromone lure can be used as an efficient bait for male trapping in Iranian corn fields. As host races exists in *O. nubilalis* (Bourguet *et al.*, 2000, Pélozuelo *et al.*, 2004), further investigations are necessary to determine if ECB populations exploiting other host plants also belong to the *Z* race or belong to the *E* race.

Further investigations should be planed to use pheromone baited WMC trap on a whole season and prove that it can be a satisfying tool for the monitoring of *O. nubilalis*. Integrating such a monitoring tool with a control method based on *Trichogramma* would constitute an efficient and environment friendly procedure for the management of this pest. As host races exists in *O. nubilalis* (Bourguet *et al.*, 2000, Pélozuelo *et al.*, 2004), attention must also be paid to the identification of the sex pheromone from ECB populations exploiting other host plants than corn.

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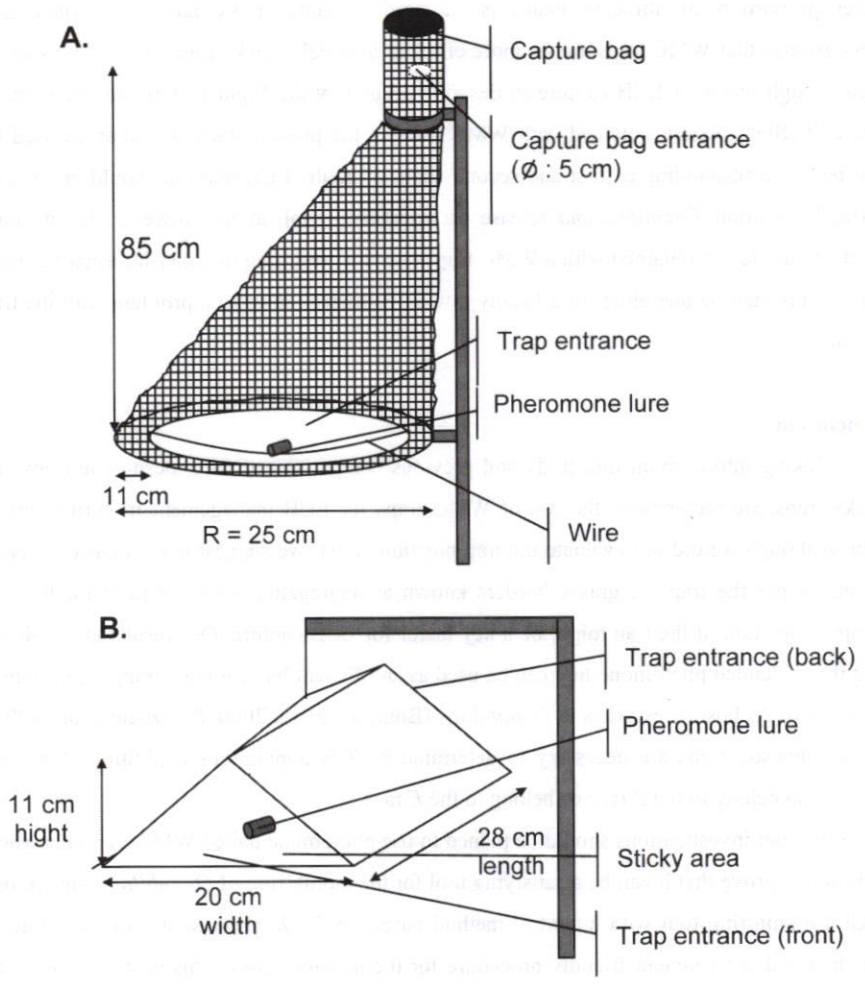

Fig. 1- Traps design used for male European Corn Borer capture. (A): home made wire mesh cone trap, (B): commercial delta sticky trap provided by NPP Calliope (France).

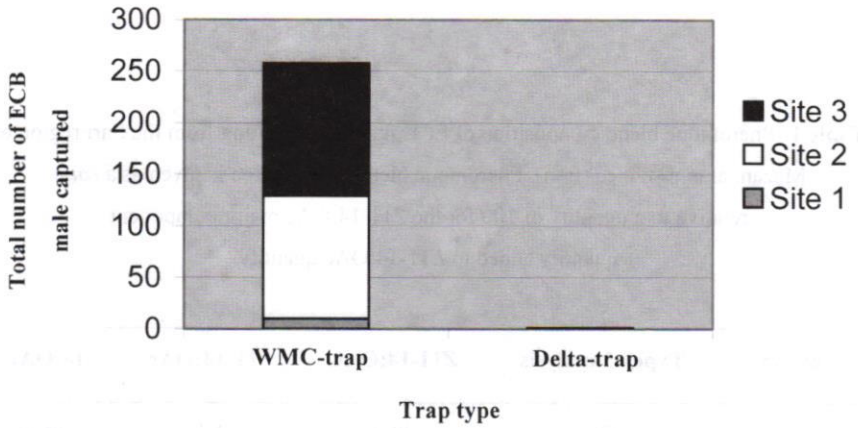

Fig. 2- Total capture of male European Corn Borer obtained with three delta sticky traps and three wire mesh cone traps in Iran (Mazandaran province, Sari region). Trapping period from July 7th 2003 to August 10th 2003.

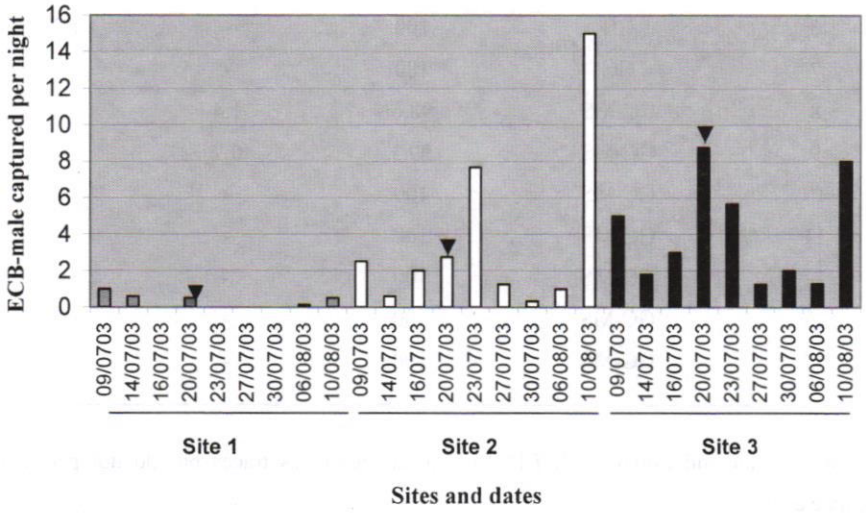

Fig. 3- Temporal pattern of ECB male capture with one WMC trap in three different sites located in the Sari region of the Mazandaran province, Iran. All traps are baited with a pheromone lure releasing a *Z* blend. Arrow indicates pheromone lure replacement.

Table 1- Pheromone blend composition of ECB female originating from the Sari region of Mazandaran province (Iran). Pheromone blend composition is given as a *ratio* relative to a quantity of 100 for the *Z*11-14:OAc major component quantity added to *E*11-14:OAc quantity.

Female	Type of analysis	*Z*11-14:OAc	*E*11-14:OAc	14:OAc
1	GC	98,5	1,5	14,9
2	GC	98,0	2,0	19,1
3	GC	100	-	17,7
4	GC	100	traces	24,5
5	GC	100	-	13,2
6	GC	100	-	8,7
7	GC	100	-	-
8	GC-MS	98,6	1,4	-
9	GC-MS	99,3	0,7	-
10	GC-MS	100	-	-
11	GC-MS	100	-	-
12	GC-MS	100	-	-
13	GC-MS	100	-	-
14	GC-MS	100	-	-

a. For the individual n° 4, *E*11-14:OAc is present as traces but do not permit to calculate a *ratio*.

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