

Short communication. Mutual pheromone antagonism in two sympatric corn borers, *Sesamia nonagrioides* and *Ostrinia nubilalis*, under field conditions

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Abstract

In previous studies, we demonstrated cross-antagonism in pheromone perception between pheromone components of the two corn (*Zea mays* L.) borers *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) in the laboratory and in the field. The two pheromone components identified as responsible for this cross-antagonism were Z 11-16:Ald, a minor component of *S. nonagrioides* pheromone, and Z 11-14:Ac, the main component of the pheromone of the Z-strain of *O. nubilalis*, which inhibited the response of *O. nubilalis* and *S. nonagrioides*, respectively. Here, we study this antagonism phenomenon in the field by air permeation of maize plots with each of the two components separately and measurement of mating in caged couples of the two corn borers on treated and untreated plots during three years. A significant reduction in mating rates was observed on the permeated plots: 7% for *S. nonagrioides* and 12% for *O. nubilalis*. When dispenser charges (200 ng) were increased by 50% and 75% in the third year, no decrease in mating rates was recorded at either of the increased concentrations. On the other hand, the use of large cages resulted in an increase of 8% to 12% in the percentage of unmated females in each of the two corn borers suggesting that at more realistic field corn borer densities, 0.1 couples/plant instead of the 2 couples/plant used in this experiment, cross-antagonism in the two corn borers could be higher than that recorded in small cages.

Additional key words: inhibition; maize; mating disruption.

Resumen

Comunicación corta. Antagonismo mutuo entre las feromonas de dos taladros simpáticos, *Sesamia nonagrioides* y *Ostrinia nubilalis*, en condiciones de campo

En trabajos previos mostramos el antagonismo cruzado en la percepción feromonal de dos taladros del maíz (*Zea mays* L.), *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae) y *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) en el laboratorio y en experimentos de campo. Los dos componentes feromonales identificados como responsables de este antagonismo cruzado fueron Z 11-16:Ald, componente minoritario de *S. nonagrioides* y Z 11-14:Ac, el componente principal de la raza Z de *O. nubilalis*, que inhibieron la respuesta de *O. nubilalis* y *S. nonagrioides*, respectivamente. Ahora hemos estudiado este mismo fenómeno en el campo a lo largo de tres años impregnando el aire de las parcelas con cada uno de los componentes citados y midiendo el apareamiento de parejas enjauladas de cada especie en parcelas tratadas y no tratadas. Se observó una reducción del porcentaje de apareamientos en las parcelas tratadas, 7% para *S. nonagrioides* y 12% para *O. nubilalis*. Cuando la carga de los difusores (200 ng) se incrementó en un 50% y un 75% durante el tercer año el porcentaje de apareamiento no disminuyó. Por otro lado, el uso de jaulas grandes supuso un incremento del 8 al 12 % en el porcentaje de hembras no apareadas en cada una de las dos especies de taladros. Esto sugiere que para densidades de campo más realistas, 0,1 parejas/planta, en lugar de 2 parejas/planta utilizadas en este experimento, el antagonismo cruzado en los dos taladros puede ser mayor que el encontrado en las jaulas pequeñas.

Palabras clave adicionales: confusión sexual; inhibición; maíz.

Maize (*Zea mays* L.) yield in several countries of the Mediterranean basin is affected by the Mediterranean corn borer, *Sesamia nonagrioides* Lefèbvre,

in addition to the more ubiquitous European corn borer, *Ostrinia nubilalis* Hübner (Eizaguirre *et al.*, 2004). The second flight and partially also the third flight of both species coincide and therefore adults of the two species can interact through their pheromone systems.

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In a previous paper (Gemeno *et al.*, 2006) we recorded cross-antagonism in pheromone perception between pheromone components of the two corn borers. In wind tunnel and electroantennogram studies, we observed behavioural inhibition of *O. nubilalis* male response to its own pheromone when the latter was mixed with a certain amount of a *S. nonagrioides* pheromone component, Z 11-16:Ald. Conversely, response of *S. nonagrioides* males was inhibited when a component of the pheromone of the other corn borer, Z 11-14:Ac was added to the pheromone blend (Eizaguirre *et al.*, 2007). This mutual behavioural antagonism was also confirmed in the field since male catches of each corn borer were practically zero when the above-mentioned components were cross-added to pheromone traps.

In order to confirm cross-behavioural antagonism between *S. nonagrioides* and *O. nubilalis* pheromone components in the field and its role in mating prevention, the effect of the two most active components as pheromone antagonists recorded in the laboratory was measured in the field. For this, the antagonistic effect of the *S. nonagrioides* pheromone Z 11-16:Ald on *O. nubilalis* mating and of the *O. nubilalis* pheromone Z 11-14:Ac on *S. nonagrioides* mating, male response was tested in the field.

Insects of *S. nonagrioides* and *O. nubilalis* used in the experiments came from the continuous rearing maintained in the laboratory and periodically renewed with wild individuals collected in the area study following the methods detailed in Eizaguirre and Albajes (1992) and Riba *et al.* (2005) respectively.

Trials were carried out during three consecutive years (2006 to 2008) in Lleida area (Catalonia, NE Spain). Treatments were replicated three times in a randomized complete block design (Gómez and Gómez, 1984). Plot size was 1500 m²; the treatment consisted of air permeation with Z 11-16:Ald or Z 11-14:Ac, placing 75 solid dispensers (one per 5 × 5 m²) and it was compared with an untreated plot. In the case of Z 11-16:Ald, the dispenser was a cylinder of PETPAI (PET051[®], of 45 mm length and Ø13 mm with a cylindrical handle of 15 mm length and Ø8 mm for dispenser manipulation) and for Z-11-14:Ac, the dispenser was a bag of KVT (KVT ON[®]; size 5 × 5 cm²). Both dispensers were provided by *Sociedad Española de Desarrollos Químicos*, SEDQ[®], Barcelona, Spain. Dispensers were charged with 200 ng of each pheromone component. They were tied to a maize leaf at 1.2 m height. The evaluation period covered 6-7 weeks from approximately twelfth-fourteenth leaf fully emerged

to silking corn (V12-14 to R1, in Ritchie's nomenclature) (Ritchie *et al.*, 1992).

Three 1- to 2-day-old virgin couples of *S. nonagrioides* or *O. nubilalis* were introduced into the cages [made with a wooden frame (0.5 × 0.4 m² and 0.5 m high) and covered with a plastic screen (mesh 2 × 2 mm²)] and these were placed on plots permeated with Z 11-14:Ac or Z 11-16:Ald, respectively and on the untreated plots. Couples were kept in cages for 2 to 3 days and then removed. The females were brought to the laboratory to assess mating status by observing spermatophore presence.

In order to improve the evaluation technique, influence of cage size on mating rates was studied by comparing the cages described above with larger ones (2.35 × 0.6 m² and 0.7 m high). The total number of *S. nonagrioides* and *O. nubilalis* females recovered from cages and then dissected was in 2006 287/344, in 2007 208/242 and in 2008 454/383, respectively. Additionally, the influence of the pheromone component amount on mating rates was evaluated by increasing the concentration in dispensers by 50 and 75%. These two treatments were added to each block in 2008.

The pheromone released throughout the experimental period has been measured each year by analysis of the dispensers at the beginning and at the end of the experimental period. A total of 16 dispensers (8 loaded with each component) were placed to monitor release rate throughout the experimental period. The amount of active ingredient in the dispensers at the moment of installation in the field and the content of each pheromone component remaining in the 16 dispensers at the end of the experimental period (45 days after deployment in the field) were recorded. Extraction of each group of dispensers was performed in a Soxhlet apparatus using 200 mL of hexane at reflux for 10 h. Quantification was carried out using a HP-1 12 m × 0.2 mm × 0.33 mm capillary column with nonyl acetate as internal standard under the following chromatographic conditions: injection at 50°C and program of 10°C min⁻¹ up to 270°C, which was maintained for 10 min. The total amount released during the experimental period was calculated by subtracting the mean amount remaining in the dispensers at day 45 from the amount found at day 0.

To analyse the efficacy of the treatment for each corn borer, we calculated the number of mated and unmated females during the year and the mean percentage of unmated females [$100 \cdot (\text{unmated}) / (\text{unmated} + \text{mated})$] transformed by $\text{ASIN}[\text{SQRT}(x100)]$ to

Table 1. Percentages (\pm SE) of unmated females of the two corn borers in cages placed in plots treated with the *O. nubilalis* pheromone component (Z)-11-tetradecenyl acetate (Z 11-14:Ac) (against *S. nonagrioides*) and the *S. nonagrioides* pheromone component (Z)-11-hexadecenal (Z 11-16:Ald) (against *O. nubilalis*). The number of values used to calculate means is shown between brackets. Means within each corn borer species followed by different letters are significantly different according to the ANOVA results shown

Treatment	<i>Sesamia nonagrioides</i>	<i>Ostrinia nubilalis</i>
ANOVA	F = 19.97; df = 1,6; $p = 0.04$	F = 24.99; df = 1,6; $p = 0.03$
Treated	33.34 \pm 6.05 (9) ^a	56.51 \pm 5.76 (9) ^a
Untreated	25.28 \pm 4.20 (9) ^b	44.21 \pm 4.33 (9) ^b

normalise it. A split-plot-like model (Gómez and Gómez, 1984) was used to analyse this percentage. Year was considered the main plot and treatment the subplot. All factors, except blocks, were considered fixed. Influence of cage size and pheromone component concentration on mating rate was analysed with a three-way (cage size, component concentration and disruption treatment) ANOVA and LSD test to compare means. Mean corrected efficacy during the experimental period was calculated with Schneider-Orelli's formula (Püntener, 1981): [corrected efficacy = 100* (% response in treatment – % response in control)/(100 – % response in control)].

The percentage of unmated females was significantly higher for both corn borers in the treated plots, as shown in Table 1 regardless of the year. Corrected mean efficacy of antagonism at the end of the experiment was 10.8% for *S. nonagrioides* and 20.0% for *O. nubilalis* in base to the percentages of unmated females shown in Table 1.

Larger cages showed significantly higher percentages of unmated females in both corn borer species (Table 2), with an increase of about 12% for *S. nonagrioides* and about 8% for *O. nubilalis*. On the contrary, increases of 50% and 75% in the pheromone component concentration did not significantly decrease ma-

ting rates (F = 0.00; df = 2,21; $p = 0.99$ for *S. nonagrioides* and F = 1.64; df = 2,21; $p = 0.22$ for *O. nubilalis*).

The total amount of pheromone components released during the experimental period is shown in Figure 1. In the case of Z 11-14:Ac to prevent *S. nonagrioides* mating, the amount actually released was 80 g ha⁻¹. In the case of the Z 11-16:Ald component to prevent *O. nubilalis* mating, the values were close to 80 g/ha in 2006 and 2008 but clearly lower in 2007.

Over three years of study in the field, the recorded mutual antagonism of pheromone components to male response in the two corn borers has confirmed the results previously obtained in the laboratory. The earlier studies had shown that when Z 11-16:Ald, a pheromone component of *S. nonagrioides* pheromone, was added to the *O. nubilalis* pheromone blend, it significantly reduced oriented flight and pheromone source contact in the wind tunnel of *O. nubilalis* males and nearly eliminated trap catch in the field (Gemeno *et al.*, 2006). A similar antagonistic response was recorded in the male of *S. nonagrioides* when Z 11-14:Ac, the main component of local populations of *O. nubilalis* (Sans, 1996), was added to a pheromone blend of the former (Eizaguirre *et al.*, 2007).

The results of the present work show that the release of the *O. nubilalis* pheromone component reduced

Table 2. Percentages (\pm SE) of unmated females of the two corn borers in small or large cages (see M&M section) placed in plots treated with *O. nubilalis* pheromone component (Z)-11-tetradecenyl acetate (Z 11-14:Ac) (against *S. nonagrioides*) and *S. nonagrioides* pheromone component (Z)-11-hexadecenal (Z 11-16:Ald) (against *O. nubilalis*) and on untreated plots. The number of values used to calculate means is shown in brackets. Within each treatment and species, means followed by different letters are significantly different according to the ANOVA results shown

	<i>Sesamia nonagrioides</i>		<i>Ostrinia nubilalis</i>	
Treated	35.67 \pm 2.29 (18) ^a	F = 5.94; df = 1,23; $p = 0.02$	73.18 \pm 5.81 (15) ^a	F = 33.16; df = 1,21; $p < 0.001$
Untreated	24.33 \pm 3.86 (17) ^b		55.13 \pm 3.54 (16) ^b	
Small cage	24.46 \pm 2.69 (18) ^b	F = 5.05; df = 1,23 $p = 0.04$	65.45 \pm 3.37 (18) ^b	F = 4.82; df = 1,21; $p = 0.04$
Large cage	36.21 \pm 4.00 (17) ^a		73.18 \pm 5.81 (15) ^a	

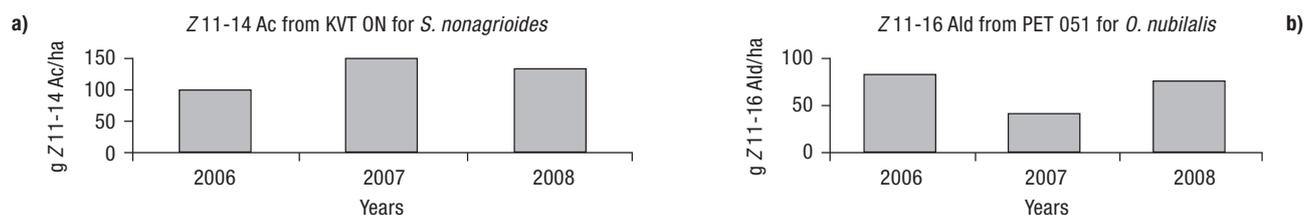


Figure 1. Amount (g ha⁻¹) released during the experimental period of the two pheromone components that were tested to prevent mating in a) *S. nonagrioides* (Z 11-14:Ac) and b) *O. nubilalis* (Z 11-16:Ald).

mating of *S. nonagrioides* couples and that the release of *S. nonagrioides* pheromone component in the field reduced mating of *O. nubilalis* couples. The efficacy of the treatment in terms of mating disruption was apparently low in comparison with other works (Perdiguer *et al.*, 1992; Albajes *et al.*, 2002; Sole *et al.*, 2008). However, as the main objective of this work was not to put a mating disruption system into practice but to confirm the cross-antagonistic action on pheromone perception by males of the two corn borers, the worst-case situation was chosen. To evaluate the potential of cross-antagonism to disrupt mating in practice, experiments should be carried out in more realistic conditions.

At least three kinds of experimental factors should be taken into account when a more realistic evaluation of cross-antagonism potential for mating disruption purposes is performed. First, common adult densities in the field are much lower than those used in our experiments within the cages. In the field, 0.1 couples per plant is a common adult density in the second generation adults whereas in this work, densities of 2 and 0.25 couples per plant, small and large cages respectively, have been tested. The lower mating rates found in females released into larger cages in comparison with smaller ones would confirm that visual recognition might play a role in high densities as it had been observed in the field (López *et al.*, 2002).

An insufficient amount of pheromone components released on the treated plots is a second experimental factor that could influence the recorded efficacy of mating disruption but it was shown in that study that an increased concentration in the dispenser charge did not lead to an increase in the antagonistic effect. Therefore, it does not seem that the low efficacy rates of mating disruption found were due to an insufficient antagonist concentration on the treated plots. In fact, Fadamiro *et al.* (1999) found similar levels of disruption when they used 240 g ha⁻¹ of the complete pheromone of *O. nubilalis* to disrupt its mating.

A third experimental factor to be considered in the evaluation of cross antagonism for mating disruption

in the field concerns with dispensers and release rate. Improving release rates and uniformity of dispensers should be one of the objectives of the system.

In summary, the capacity of some of the pheromone components of each of the two corn borers as behavioural antagonists of the other has been confirmed in the field, as it has been observed in the laboratory. Such antagonistic behaviour may be used simultaneously for mating disruption of the two corn borers, although it may be expected that mating in *O. nubilalis* was more efficiently disrupted than in *S. nonagrioides*. However, for practical applications of cross-antagonism in the field for mating disruption, release rates of dispensers, concentration of antagonist in the dispenser and techniques to be used to assess efficacy should be further investigated.

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References

- ALBAJES R., KONSTANTOPOULOU M., ETCHEPARE O., EIZAGUIRRE M., FRÉROT B., SANS A., KROKOS F., AMÉLINE A., MAZOMENOS M., 2002. Mating disruption of the corn borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae) using sprayable formulations of pheromone. *Crop Prot* 21, 217-255.
- EIZAGUIRRE M., ALBAJES R., 1992. Diapause induction in the stem corn borer, *Sesamia nonagrioides* (Lepidoptera, Noctuidae). *Entomol Gen* 17, 277-283.
- EIZAGUIRRE M., LÓPEZ C., ALBAJES R., 2004. Dispersal capacity in the Mediterranean corn borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Entomol Exp Appl* 113, 25-34.

- EIZAGUIRRE M., ALBAJES R., LÓPEZ C., SANS A., GEMENO C., 2007. Inhibition of pheromone response in *Sesamia nonagrioides* by the pheromone of the sympatric corn borer *Ostrinia nubilalis*. *Pest Manag Sc* 63, 608-614.
- FADAMIRO H.Y., COSSE A.A., BAKER T.C., 1999. Mating disruption of European corn borer *Ostrinia nubilalis* by using two types of sex pheromone dispensers deployed in grassy aggregation sites in Iowa cornfields. *J Asia Pacific Entomol* 2, 121-132.
- GEMENO C., SANS A., LÓPEZ C., ALBAJES R., EIZAGUIRRE M., 2006. Pheromone antagonism in the European corn borer moth, *Ostrinia nubilalis*. *J Chem Ecol* 32, 1071-1084.
- GÓMEZ K.A., GÓMEZ A.A., 1984. Statistical procedures for agricultural research, 2nd ed. Wiley, NY, USA. 680 pp.
- LÓPEZ C., EIZAGUIRRE M., ALBAJES R., 2002. Courtship and mating behaviour of the Mediterranean corn borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Span J Agric Res* 1(1), 43-51.
- PERDIGUER A., GIMENO F., AGUILAR L., EIZAGUIRRE M., RIBA, M., SANS A., 1992. Ensayos de confusión sexual en *Sesamia nonagrioides*. *Invest Agrar: Prod Protec Veg* 7, 253-260. [In Spanish].
- PÜNTENER W., 1981. Manual for field trials in plant protection second edition. Agricultural Division, Ciba-Geigy Limited, Basle, Switzerland.
- RIBA M., SANS A., SOLÉ J., MUÑOZ L., BOSCH P., ROSELL J.A., GUERRERO A., 2005. Antagonism of pheromone response of *Ostrinia nubilalis* males and implications on behavior in the laboratory and in the field. *J Agric Food Chem* 53, 1158-1165.
- RITCHIE S.W., HANWAY J.J. BENSON G.O., 1992. How a corn plant develops. Special Report n. 48. Iowa State University, Ames, USA.
- SANS A., 1996. Les feromones sexuals en el control dels barrinadors del blat de moro *Ostrinia nubilalis* Hbn. i *Sesamia nonagrioides* Lef. Ph D dissertation. University of Lleida, Lleida, Spain. [In Catalan].
- SOLÉ J., SANS A., RIBA M., ROSA E., BOSCH M.P., BARROT M., PALENCIA J., CASTELLÀ J., GUERRERO A., 2008. Reduction of damage by the Mediterranean corn borer, *Sesamia nonagrioides*, and the European corn borer, *Ostrinia nubilalis*, in maize fields by a trifluoromethyl ketone pheromone analog. *Entomol Exp Appl* 126, 28-39.