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Mating disruption to control the striped rice stem borer: pheromone blend, dispensing technology and number of releasing points

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Abstract

The effect of using low densities of different dispensing technologies on mating disruption of the striped rice stem borer, *Chilo suppressalis* Walker, was evaluated in the rice-growing area of Valencia (Spain) from 2011 to 2013. The reduction of the current number of release points (30 polymeric dispensers/ha) was investigated by installing 3 aerosol devices per ha (Experiments 1 and 2) or with clusters of hand-applied dispensers (10 or 5 release points/ha; Experiment 3). The influence of pheromone blend on disruption was also studied by loading aerosol devices with the three-component blend or only the main pheromone compound, *Z*-11-hexadecenal. Results showed that the installation of 3 aerosol devices/ha or clusters of passive dispensers (total dose: 6.6-7.9 g/ha) proved equally effective as the conventional treatment with 30 Selibate®CS dispensers/ha (~5g/ha), reducing damage below 1% of infested plants. Although the treatment with 3 aerosol devices/ha loaded with *Z*-11-hexadecenal provided control of damage comparable to the conventional mating disruption treatment, the higher captures recorded suggest that mating disruption with the incomplete pheromone blend is only slightly effective in the tested conditions. These changes in the number of point sources and pheromone blend could represent important advantages for the implementation of mating disruption against *C. suppressalis*.

Keywords

Chilo suppressalis; aerosol dispensers; mesoporous dispensers; integrated pest management; paddy fields

Introduction

Striped rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae), is a key rice pest that is widely distributed in most temperate areas of Asia (China, India, Indonesia, Iran, Japan, Korea, the Philippines) and Europe (Spain, France, Portugal and Hungary). It has also been detected in Russia, Hawaii (USA) and the northern territories of Australia (EPPO, 2014). *C. suppressalis* can develop up to five generations in the most temperate regions (Hou et al., 2010), but in our study area (Spanish Mediterranean coast) the pest has three male flights peaking on (1) the beginning of June, (2) July-mid August, and (3) the beginning of September. The larvae of rice borers feed within plant stems, causing severe crop loss in many cases (Beevor et al., 1990; Batalla, 1999). Thus, its concealed nature makes the control of *C. suppressalis* with foliar contact insecticides difficult (Beevor et al., 1990; Howse, 1998). Currently in Spain, *C. suppressalis* is being controlled by using insect growth regulators and mating disruption or mass trapping methods, especially in environmentally protected areas.

C. suppressalis pheromone was first identified as the aldehyde blend containing (*Z*)-11-hexadecenal (*Z*11-16:Ald) and (*Z*)-13-octadecenal (*Z*13-18:Ald) (Nesbitt et al., 1975; Ohta et al., 1976). It was later demonstrated that the attractant power of this blend was less efficient than that of virgin females. Thus, the pheromone composition was revised and completed with a third aldehyde, (*Z*)-9-hexadecenal (*Z*9-16:Ald) (Tatsuki et al., 1983). Attractant activity significantly increased with this 3-component blend in an approximate ratio of 48:6:5 (*Z*11-16:Ald / *Z*13-18:Ald / *Z*9-16:Ald) (Beevor et al., 1990). After it was first identified in the late 1970s, several studies demonstrated that *C. suppressalis* sexual communication could be disrupted with its sex pheromone or other structurally related compounds, and that a good level of inhibition of attraction, mating and infestation suppression could be obtained with only the major pheromone component, *Z*11-16:Ald (Kanno et al., 1982). Depending on the mechanisms responsible for the mating disruption, best efficacy should be achieved when the disruptant closely matches the pheromone emitted by females, but successful disruption might also be attained through the release of a partial pheromone blend (Cardé and Minks, 1995; Witzgall et al., 2008). The feasible use of simple pheromone blends or single chemicals would help implement these types of control methods because the synthesis of the chemicals involved may come to more than 90% of the technique's cost.

Disruption of pheromone communication can be affected by, among other factors, the amount of chemical released into the air and the spacing between the release points. Besides spray and paraffin wax formulations, there are two main ways of implementing mating disruption with pheromone-release devices: hand-applied passive pheromone dispensers spaced close together (from 100 to 10,000 per ha), or high-releasing active pheromone evaporators (aerosol dispensers) placed with more widely-spaced separations in the field (Shorey et al., 1996). Aerosol devices can protect sex pheromones from UV degradation and oxidation and can be programmed to atomize pheromone at regular intervals, which saves pheromone costs. Their use also saves labor costs during field installation as they can be placed at very low densities (1-5 units/ha), even allowing their deployment along field margins or perimeters. Although the mating disruption of *C. suppressalis* has always been approached with passive polymer formulations, field experiments conducted in Spain have managed to increase the distance between dispensers from 2 m (2,500 dispensers/ha) to 16 m (39 dispensers/ha) (Casagrande, 1993; Alfaro et al., 2009). This is especially important for rice fields as the installation of multiple dispensers evenly distributed in a grid involves going into muddy paddy fields. Thus, a mating disruption strategy with 3-5 active diffusion dispensers per ha would allow device placement using plot margins, which is a great advantage to implement this technique.

The main aim of the present work was to explore the efficacy of mating disruption against *C. suppressalis* using low densities of different pheromone dispensing technologies and the effect of incomplete pheromone blends (only the main compound, *Z*11-16:Ald). Mating disruption treatments with experimental aerosol devices were deployed in different years to test the approach of sparse pheromone sources in preventing injury to rice plants. The reduction on pheromone release points was also investigated with passive low-releasing dispensers. The influence of the pheromone blend on disruption was studied by loading aerosol dispensers with the three-component pheromone blend and *Z*11-16:Ald alone.

Materials and methods

Pheromone dispensers

Saturel® mesoporous dispensers. This type of passive dispensers is based on the technology using inorganic molecular sieves developed by Corma et al. (1999, 2000), with a patent licensed to Ecología y Protección Agrícola SL (Valencia, Spain). The dispenser matrix is sepiolite, a natural clay mineral with high adsorptivity for organic molecules. These dispensers are cylindrical tablets loaded with 250 mg/dispenser of the three-component pheromone blend, *Z*11-16:Ald / *Z*9-16:Ald / *Z*13-18:Ald (82:8:10). Saturel® dispensers were placed in the field on stakes, at 0.6 m above the ground, inside polymeric blisters, and the pheromone was released through lateral holes (Fig. 1). For Experiment 3, Saturel® dispensers were loaded only with 200 mg of the major component *Z*11-16:Ald (Saturel®-M).

Selibate®CS dispensers. These are the commercial cylindrical rubber passive dispensers supplied by Suterra Europe Biocontrol SL (Valencia, Spain), loaded with 400 mg of the three-component pheromone blend, *Z*11-16:Ald / *Z*9-16:Ald / *Z*13-18:Ald (84:10:6). Individual dispensers were placed in the field on stakes, at 0.6 m above the ground.

Neburel® devices. These are experimental mechanical devices used to apply aerosol pheromone formulations to allow the active release of pheromone at programmed time intervals. They were provided by Ecología y Protección Agrícola SL (Valencia, Spain) and consist of a plastic cabinet that houses the electronic timer, the actuator, the batteries and the aerosol canister containing the pheromone blend. Two types of formulations were tested in Experiments 1 and 2: Neburel®-Z, loaded with 2.50 g per canister of the three-component pheromone blend, *Z*11-16:Ald / *Z*9-16:Ald / *Z*13-18:Ald (82:8:10). The second was Neburel®-M, loaded only with 2.05 g per canister of the major pheromone component, *Z*11-16:Ald. Emitters were placed on stakes, at 1 m above the ground.

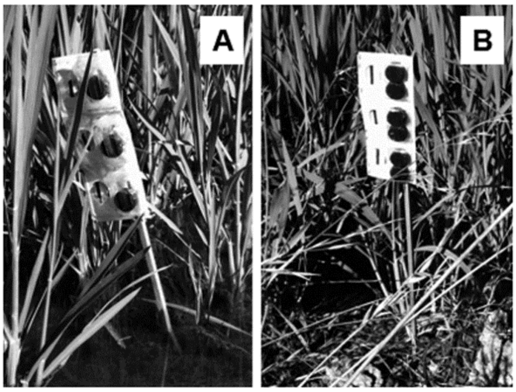

Fig. 1 Saturel® dispensers employed in Experiment 3 (2013): (A) 6 Saturel®-M: 3 units of mesoporous dispensers on each side of the blister; (B) 12 Saturel®-M: 6 units of mesoporous dispensers on each side of the blister.

Field trials

The trials included in the present work were conducted in the municipalities of Cullera and Favara, within the environmentally protected area of the Albufera Natural Park (Valencia, Spain) (39° 19′ 54″ N, 0° 21′ 8″ W). The 16,000 ha rice-growing area of the Albufera has been entirely treated with mating disruption since 2009, where 30 Selibate®CS dispensers/ha were installed evenly each growing season. As no untreated fields can be left for comparisons, the aforementioned conventional mating disruption treatment was employed as a reference or control. In order to avoid pheromone drift and the edge effect associated with small plots, large scale pheromone trials were performed and all the pheromone treatments tested were applied over wide areas (≥ 50 ha). Then, mating disruption efficacy was assessed at three plots within each treated area. All treatments were applied before the second *C. suppressalis* male flight, which takes place in Valencia throughout July. The arrangement of treatments in the field is depicted in Fig. 2, and Table 1 shows the characteristics of each strategy. The mean size of individual paddy-fields (~1 ha) allowed the placement of release devices along the margins of the fields comprised in the area treated with widely separated release points (3-10 points per ha). All trials ended 1-2 weeks before harvesting.

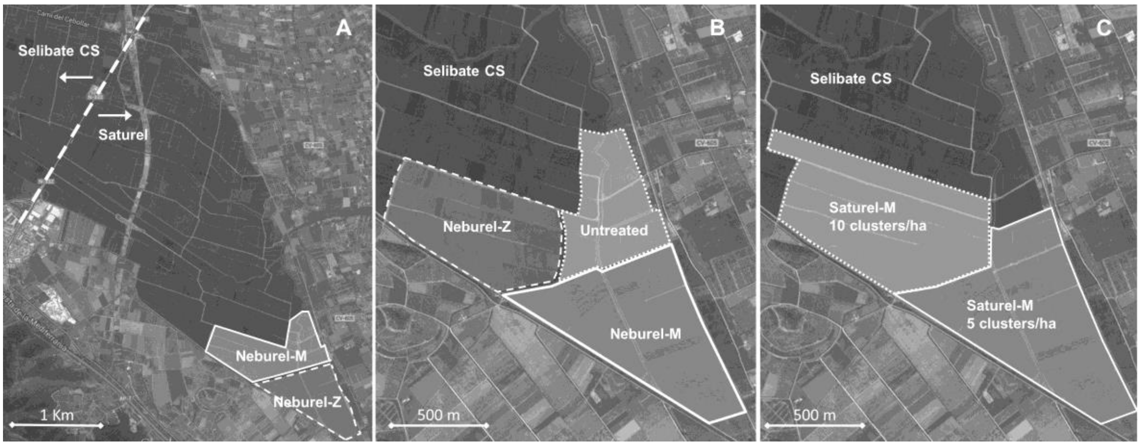

Fig. 2 Arrangement of mating disruption treatments in the different field experiments. (A) Experiment 1 (2011): Neburel®-Z and Neburel®-M (3 devices/ha), Selibate®CS and Saturel® (30 points/ha). (B) Experiment 2 (2012): Selibate®CS (30 points/ha), Neburel®-Z and Neburel®-M

(3 devices/ha), and untreated area. (C) Experiment 3 (2013): Selibate®CS (30 points/ha), Saturel®-M (5 points/ha) and Saturel®-M (10 points/ha).

Experiment 1: Dispensing technology and pheromone blend. Mating disruption treatment with Saturel® mesoporous dispensers was applied in 900-ha rice fields, in a grid with 30 releasing points/ha with two dispensers per releasing point (total 60 dispensers/ha). Neburel®-Z and Neburel®-M aerosol treatments were applied at a density of 3 devices/ha in 50-ha zones. The rest of the Albufera rice-growing area (15,000 ha) was treated in a grid with 30 Selibate®CS dispensers/ha. Nine-hundred ha of conventional treatment with Selibate®CS, in adjacent fields to those treated with Saturel® and Neburel®, were used as the reference treatment. Pheromone delivery systems were placed in the field during the first week of June and the trial ended on 13 September 2011.

Experiment 2: Pheromone blend. In 2012, the trial was conducted in the same growing area as above to confirm the results obtained with the aerosol devices during the previous trial. Mating disruption treatments with Neburel®-Z and Neburel®-M aerosol were applied in 100-ha areas at a density of 3 devices/ha. A second density of 1.5 devices/ha of Neburel®-M aerosols was also installed in another 100-ha area, but 90% of the devices were stolen during the first 15 days of the trial; thus, the remaining devices were removed and the data on this area were considered an untreated reference plot (no additional insecticide treatments were applied in this area). A conventional mating disruption treatment with 30 Selibate®CS dispensers/ha was also used as a reference treatment. Pheromone treatments were in place between the first week of June to 6 September 2012.

Experiment 3: Varying densities of pheromone release points. Smaller numbers of release points per ha, using clusters of passive dispensers (Fig. 1), were tested from June to September 2013 in the same rice-growing area. Saturel®-M dispensers, loaded only with *Z*11-16:Ald, were applied on two areas (100 ha each) to test the efficacy of the mating disruption treatment with low-releasing dispensers applied at 5 and 10 releasing/points ha, with clusters of 6 and 12 mesoporous dispensers. This maintained the total pheromone dose, but lowered the number of releasing points. Groups of dispensers were installed at the field margins. As in the above-described trials, the reference treatment was Selibate®CS (30 dispensers/ha).

Evaluation of treatment efficacy

Catch suppression efficacy attained by the different experimental mating disruption treatments was compared with the reference treatment (Selibate ®CS) by installing three (Experiments 1 and 2) or four (Experiment 3) pheromone-baited commercial funnel traps (Lepisan®, Sansan Prodesing SL, Valencia, Spain) inside each treated area. Monitoring traps were positioned at least 50 m apart from any paddy margin to avoid edge effect. Each trap was baited once with a 6-mg commercial polyethylene vial monitoring dispenser (SEDQ SL, Barcelona, Spain) and a DDVP strip as the insecticide. Captures were recorded biweekly during the trials. Absence of moth captures during mating disruption treatment is a good indication of the effectiveness of the technique, but damage assessment provides the final proof for efficacy. Evaluation of catch suppression and crop damage was carried out in the center of the treated areas to check the actual effect of each treatment minimizing all kinds of interferences, such as pheromone drift or pest intrusion.

Crop damage was assessed by counting the number of infested plants per m^2 in randomly selected central 1-m^2 plots. A 1-m^2 frame made of cane was thrown to select these plots and all the plants inside the frame were counted and inspected. Typical stem borer symptoms (dried up central shoots of tillers and discolored panicles with unfilled grains) were observed and the presence of sawdust and *C. suppressalis* life stages (larvae and pupae) inside the stems were checked to verify damage. Frame was thrown 6-8 times in three different plots separated by 250 m within each mating disruption area (ca. 24 plots of 1-m^2 assessed in the central area for each treatment). In all the experiments, assessments were conducted in mid-September, a few days before harvesting.

Pheromone emission. To verify pheromone emission rates from passive dispensers and the total pheromone doses applied, the rate of loss of pheromone was examined for the Selibate®CS and Saturel® dispensers by solvent extraction with dichloromethane and gas-chromatography (GC/FID) quantification with hexadecane as an internal standard. Additional dispensers were aged under field conditions and sampled on days 15, 45, 60, 90 to quantify the residual pheromone load contained. Three replicates per aging time were extracted from each type of dispenser. Saturel® mesoporous dispensers were extracted by soaking in solvent and magnetic agitation for 2 h, whereas the polymeric matrix of Selibate®CS was extracted by pressurized solvent extraction using the One PSE™ apparatus (Applied Separations, Bethlehem, PA, USA). Extraction conditions were 100 bar, 60ºC and nine cycle extractions of 5 min. The GC/FID analysis of all the resulting extracts used a Clarus500 gas chromatograph from PerkinElmer (Wellesley, MA, USA). All injections were made onto a ZB-5MS column (30 m by 0.25 mm by 0.25 µm; Phenomenex Inc., Torrance, CA, USA) that was held at 100ºC for 2 min and programmed at 15ºC/min to 170ºC, held at 170ºC for 5 min; then at 20ºC/min to 240ºC and held at 240ºC for 1 min. The carrier gas was helium at 1.2 ml/min. The pheromone amount was estimated according to calibration curves $y = \mathrm{a}x + \mathrm{b}$, where y is the ratio between pheromone and internal standard GC responses and x is the amount of pheromone. As loss of pheromone was constant, the average emission rate over the study period was estimated by fitting linear models (residual pheromone load (mg) vs. time (days of field exposure)). The slope of the fitted line for each pheromone component gave the estimation of the mean release rate (Table 1).

On the other hand, Neburel® aerosol devices were previously calibrated by testing different ratios of pheromone and propellant gas to obtain the desired emission rates. The gravimetric method was used to determine the amount of pheromone released in relation to time, by weighing weekly additional canisters on a precision balance. Neburel®-Z delivered 0.73 mg of the three-component pheromone blend every 30 min for at least 70 days, whereas Neburel®-M devices delivered 0.60 mg of *Z*11-16:Ald every 30 min, for at least 70 days.

Statistical Analyses

Generalized linear model (GLM) techniques assuming *quasipoisson* error variance were employed to compare the number of moths captured in the different pheromone treated plots. Moth capture data were summed across sample dates and were employed as the dependent variable to construct GLM models. Treatment, time (week of the study period) and their interaction were included in the models as the explanatory variables, whose significance was assessed by backward elimination from the model. When significant effects were found (F test), the *glht* function in the multcomp package (Hothorn et al., 2008) was used to perform Tukey HSD tests for post-hoc pairwise comparisons.

Likewise, we used GLM techniques assuming *quasipoisson* error variance to assess plant infestation differences between the different treatments. Models were constructed with the percentage of infested plants as the dependent variable and treatment as the explanatory variable. The significance of factor effects and multiple comparison tests were carried out as described above. All statistical analyses were conducted with R (R version 3.1.0) (R Development Core Team, 2014).

Results

Experiment 1: Dispensing Technology and Pheromone Blend

Captures in monitoring traps. Catches in monitoring traps showed *C. suppressalis* population dynamics in the study area, with low levels throughout the trial and the most abundant third flight peaking in September (Fig. 3A). Unfortunately, more than 30 aerosol dispensers were stolen in part of the area treated with Neburel®-M three weeks before the trial ended, which accounts for the sudden increase in moth catches recorded in this plot at the last evaluation of the monitoring traps. Accordingly, catch data of the last sampling period (16 September) was not included in the statistical analysis. The interaction between the factors studied (treatment × week) was not statistically significant ($F = 0.54$; df = 15,48; $P = 0.90$), and so consequently it was disregarded from the analysis. Results showed that moth captures were affected by the treatment applied (treatment factor: $F = 11.19$; df = 3,52; $P < 0.001$) and they were significantly higher in the area with Saturel® dispensers ($P < 0.003$, post-hoc pairwise comparisons). Effect of time (week factor) on moth captures was also significant due to the pest population dynamics itself ($F = 7.27$; df = 4,52; $P < 0.001$). Captures in the areas treated with aerosol devices were similar to those recorded in the reference plots with Selibate®CS dispensers ($P > 0.77$, post-hoc pairwise comparisons).

Crop damage. Despite the increase in catches at the end of the trial, the second male flight was clearly affected and all treatments had a low percentage of plant infestation (Fig. 3B) not differing significantly (treatment factor: F = 1.39; df = 3,8; P = 0.31). Even though moth captures were significantly higher in the area with Saturel® dispensers, level of disruption provided was still enough to control damage and both experimental mesoporous dispensers and aerosol devices were as effective as the commercial reference treatment with Selibate®CS dispensers. It must be taken into account that cumulative moth captures in the area treated with Saturel® was 3 moths per trap, a very low number of captures when compared to the level of catches (15-43 moths/trap) reported in this area when mating disruption was not yet applied (Alfaro et al., 2009).

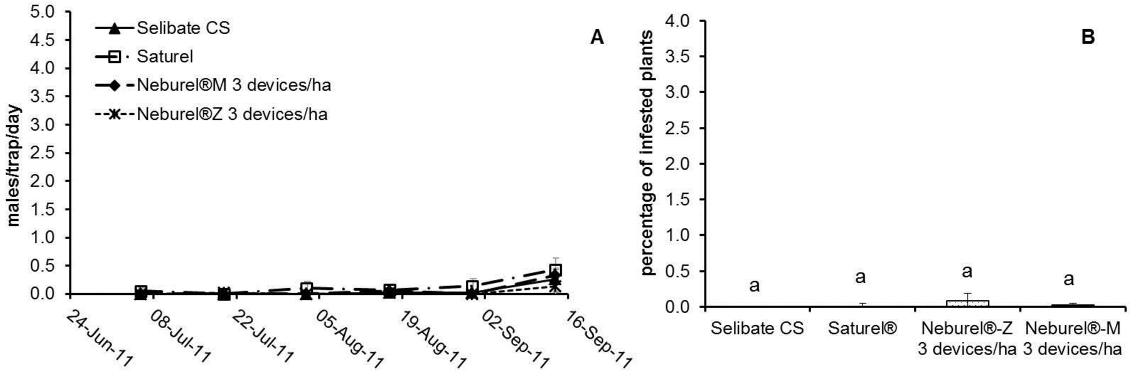

Fig. 3 Results of Experiment 1 (2011): (A) Captures of *Chilo suppressalis* (mean ± SEM males/trap/day) recorded in the monitoring traps located in each pheromone treated area; (B) crop damage (mean percentage of infested plants ± SEM) resulting from the different mating disruption treatments (bars labelled with the same letter are not significantly different; Tukey HSD test).

Experiment 2: Pheromone Blend

Based on the results obtained in Experiment 1 and the advantages of installing Neburel® devices, only these dispensers were tested against conventional Selibate®CS during the second trial.

Captures in monitoring traps. Male flight patterns were similar in 2011 and 2012, with the most important flight at the beginning of September (Fig. 4A). Again, the interaction between the factors was not significant (treatment × week: F = 0.30; df = 18, 51, P = 0.99) and consequently disregarded. The week factor was significant due to the pest's population dynamics (F = 23.78; df = 6,69; P < 0.001) and the pheromone treatment also resulted in a significant difference (F = 23.88; df = 3,69; P < 0.001). Monitoring traps in the untreated fields had significantly the highest captures compared with any pheromone treatment (P < 0.005, post-hoc pairwise comparisons). However, mean captures were significantly less throughout the trial in the rice fields treated with Selibate®CS and Neburel®-Z aerosols compared to Neburel®-M releasing only the major pheromone component (P < 0.02, post-hoc pairwise comparisons).

Crop damage. Although trap catch disruption was significantly better with the aerosol devices releasing the three-component pheromone blend (Neburel®-Z) than with only the major (Neburel®-M) (Fig. 4A), damage assessment conferred final proof for crop protection, highlighting that the level of plant infestation was not significantly different between the different mating disruption strategies (Fig. 4B). Only the damage observed in the untreated area was different (F = 5.35; df = 3,7; P = 0.03). Crop damage was below 0.4% of infested plants with mating disruption treatments and ca. 2% in the untreated area.

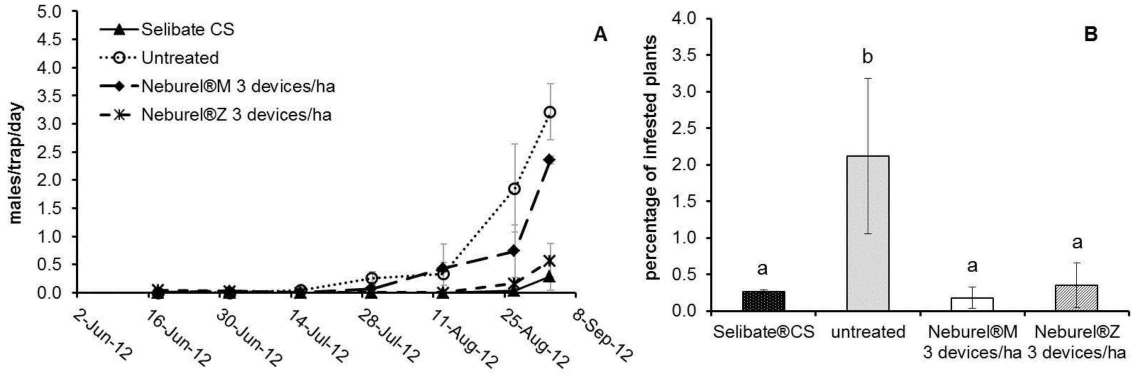

Fig. 4 Results of Experiment 2 (2012): (A) Captures of *Chilo suppressalis* (mean ± SEM

males/trap/day) recorded in the monitoring traps located in each pheromone treated area; (B) crop damage (mean percentage of infested plants ± SEM) resulting from the different mating disruption treatments (bars labelled with the same letter are not significantly different; Tukey HSD test).

Experiment 3: Varying Densities of Pheromone Release Points

Captures in monitoring traps. The population dynamics in the third trial was similar to those reported in Experiments 1 and 2. Although catch was low up to the end of August with the three strategies tested (Fig. 5A), male catches increased at the end and were significantly lower with Selibate®CS (treatment factor: F = 32.97; df = 2,88; $P < 0.001$; and the week factor: F = 85.69; df = 8,88; $P < 0.001$; but their interaction was not significant: P = 0.95).

Crop damage. The orientation disruption obtained with sparse pheromone sources of Saturel® dispensers proved sufficient to control plant infestation (Fig. 5B), which did not significantly differ from the results obtained with the reference treatment Selibate®CS (F = 1.51; df = 2,9; P = 0.27). In fact, damage was found in hotspots (only in 6 of the 24 plots assessed) in the area treated with 10 release points of Saturel® per ha. Moreover, 55% of the infested plants detected were found in only one of these plots, probably corresponding with the loss of some of the closest pheromone sources due to external factors such as wildlife (wading birds) or cultural practices (tractors).

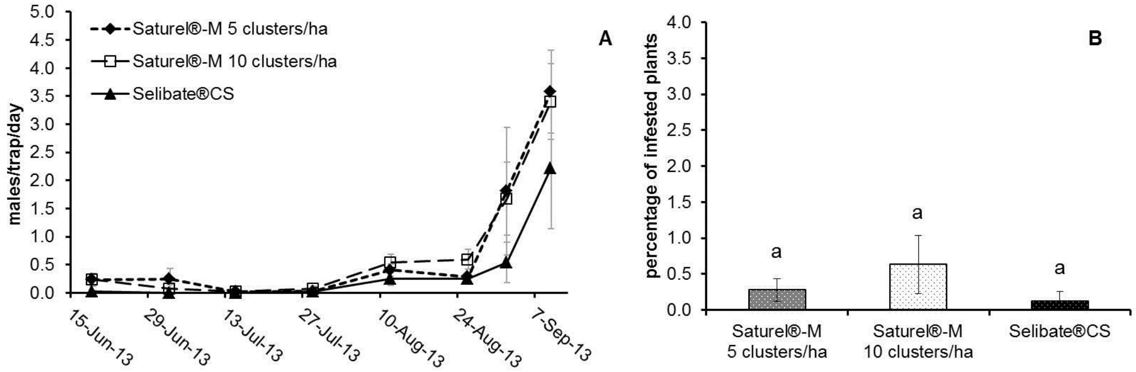

Fig. 5 Results of Experiment 3 (2013): (A) Captures of *Chilo suppressalis* (mean ± SEM males/trap/day) recorded in the monitoring traps located in each pheromone treated area; (B) crop damage (mean percentage of infested plants ± SEM) resulting from the different mating disruption treatments (bars labelled with the same letter are not significantly different; Tukey HSD test).

Discussion

Experiments by Kanno et al. (1982) confirmed that male flight can be 90% inhibited by emitting 50 mg/ha/day of the major component of the *C. suppressalis* pheromone (*Z*11-16:Ald) from polyethylene-tube dispensers 16 m apart (39 per ha). Moreover, Tatsuki (1990) obtained control by releasing 1.2-1.6 g/ha/day of the major component, reducing infestation by 77%, even with a high population density (4-7% of infested stems before treatments). Unfortunately, there are only a few studies reporting field damage assessment in the literature available. Chen et al. (2012) evaluated plant damage by selecting 800 tillered rice plants at random from a pile of unknown number of harvested plants from each trial plot, which is neglecting the number of previously fallen attacked plants that were not harvested. Only a few works report crop damage as number of affected stems measured directly in the field (Tatsuki, 1990; Serrano et al., 1998; Alfaro et al., 2009), probably due to the extremely laborious effort of inspecting stems inside rice paddies. In our study, we assessed plant damage in 24 small plots of 1-m^2, similar to the inspection carried out by Alfaro et al. (2009) in 30 small plots. In our case, all the stems inside 8 randomly selected small plots of 1-m^2 were inspected in three different points of each treated area, which represents more than 25.000 plants inspected per treated area.

The trials reported in the present work suggest that the installation of three aerosol devices per ha loaded with the complete pheromone blend (Neburel®-Z) or only *Z*11-

16:Ald (Neburel®-M) can protect rice plants from striped stem borer infestation (< 0.1% of affected plants), which is comparable to the conventional mating disruption treatment with passive polymeric dispensers. In Experiment 2, the area treated with Neburel®-M obtained higher catches than Neburel®-Z, which is suggesting that male disruption effect could be weaker by releasing only the main pheromone compound. In spite of this, damage assessment showed that the *Z*11-16:Ald emission of Neburel®-M was sufficient to control damage (0.18% infested plants), as it differed significantly from the damage recorded in the area with no control measures (2.12 % infested plants). Adult captures increased significantly in the untreated area in the period 13-27 August and these adults developed a generation that caused detectable damage in the assessment carried out in 6 September. However, given that adult captures in Neburel®-M area increased significantly only 1 week before damage assessment, the resulting generation did not have enough time to develop and cause detectable damage. This might explain why the significant higher captures were not finally reflected in higher crop damage. Nevertheless, the increasing captures could be suggesting that mating disruption with *Z*11-16:Ald is only slightly effective and it is not totally avoiding encounters but only delaying it. Use of the major pheromone component has proven efficient for other moth pests, such as codling moth (*Cydia pomonella* L.). Codlemone ((*E,E*)-8,10-dodecadien-1-ol) is the main codling moth sex pheromone compound. However, it has been shown that addition of both dodecan-1-ol and tetradecan-1-ol is necessary to obtain an equivalent close-range response to that elicited by the natural pheromone (Bartell et al., 1988). Adding synergists to codlemone may intensify the effect of mating disruption treatments by increasing male attraction and by prolonging close-range behavior near dispensers, but the role of dodecan-1-ol and tetradecan-1-ol is still unclear (Knight, 1995; Witzgall et al., 2008). Nevertheless, commercial *C. pomonella* mating disruption formulations are diverse, and the active ingredient contained (only codlemone or mixture of alcohols) depends on the manufacturer (Angeli et al., 2007; Stelinski et al., 2007; Knight and Light, 2014).

Several trials were performed from 1987 to 1990 in the rice-growing area of the Albufera, to design the most suitable mating disruption strategy (Beevor et al., 1990; Serrano et al., 1998; Batalla, 1999). Consequently, the number of passive dispensers per ha has been progressively reduced to 100 Selibate®CS, with a total pheromone quantity of 40 g/ha (Casagrande, 1993). The dispenser density eventually was lowered to 39 Selibate®CS dispensers/ha (~15 g/ha). Furthermore, Alfaro et al. (2009) reported that even a density of 16 dispensers/ha is capable of protecting rice fields against *C. suppressalis* where mating disruption has been applied for a decade. As the number of releasing points per ha does not seem to be a crucial factor, provided that the total amount of pheromone is sufficient to disrupt communication, aerosol devices may be good candidates to protect rice fields. The field trials carried out in 2011 and 2012 reported in the present work evidence this. While treatment with 30 Selibate®CS dispensers/ha is effective, with a total emitted amount of ~5g/ha of the three-component pheromone blend, the installation of 3 aerosol/ha proved equally effective when 6-7.4 g/ha were applied (*Z*11-16:Ald or complete blend). The advantages offered by aerosol devices are evident as far as the installation and the protection of the active ingredients are concerned. However, paddy fields are open, and are not usually protected by fences. Loss of units (cabinets or batteries) during the season, as occurred in our trials, is a major problem. For this reason, the mating disruption strategy using spaced pheromone sources was also tested in our third trial with clusters of passive pheromone dispensers. The placement of 5 or 10 clusters of Saturel® dispensers per ha proved as effective as the conventional treatment with 30 release points/ha, but avoids having to install stakes inside the paddy fields, consequently reducing hand-labor costs of dispenser installation. Given that pheromone represents more than 90% of the dispensers' cost, the use of passive dispensers or aerosol devices does not suppose a significant economic difference (40 €/ha for 39 Selibate®CS dispensers/ha vs. 39 €/ha for 3 Neburel®/ha). However, the cost of conventional dispenser installation is 8-10 €/ha, meanwhile the cost of installing 3 releasing points/ha would not exceed 5 €/ha.

Our work provides experimental evidence that employing sparse pheromone sources to apply mating disruption against *C. suppressalis* is efficacious provided that total pheromone dose is maintained in the environment. However, other approaches to optimization of mating disruption use response surface modelling (Lapointe et al., 2011; Willett *et al*., 2015), allowing the examination of multiple interrelated variables and not focusing on one factor at a time. These authors found that trap catch disruption declined exponentially as the degree of aggregation and distance between pheromone sources increased, by varying all factors at a time. We demonstrate in our experiments that strategies tested could be equally effective but decisions about how to implement mating disruption might be better supported by more exhaustive methods.

Disruption of pheromone communication has almost become the only control method for *C. suppressalis* in environmentally protected rice-growing areas. These treatments need to be cost-effective and several issues can be optimized for this purpose. Although results reported in this work suggest that disruption effect of *Z*11-16:Ald is weaker than with the complete pheromone blend, further trials would help clarifying the potential of the incomplete blend, such as testing the effect of higher *Z*11-16:Ald doses. The possibility of employing only the major pheromone component is a great advantage because the pheromone synthesis costs can be substantially reduced. Several authors have suggested that both the prolonged use of mating disruption (14 to

16 years after treatment started) and the use of incomplete pheromone mixtures may lead to resistance (Mochizuki et al., 2002; Tabata et al., 2007). It is suggested that mating disruption with incomplete mixtures could impose strong selection pressure on the targeted pest and induce evolutionary changes but it is not clear if this could result in effective resistance to this control technique, given that the use of the complete pheromone blend is able to restore control of the ‘selected population’ (Mochizuki et al., 2002).

Use of mechanical aerosol dispensers has proven effective for rice crops, characterized by wide, regular-shaped fields of flat terrain. On the other hand, aerosol devices (cabinets and canisters) have a relatively high cost and are vulnerable to vandalism. Nevertheless, the results reported herein suggest that aerosol release devices can be replaced with clusters of passive dispensers to generate similar high emission point sources.

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Tables

Table 1 Characteristics of mating disruption strategies tested in field trials

	treatment[a]	area (ha)	# releasing points per ha	# devices per ha	pheromone emitted (g/ha)[b]	mean release rate (mg/day/ha)[c]
2011	Saturel®	900	30	60	6.6	88.5
	Neburel®-Z	50	3	3	7.9	105
	Neburel®-M	50	3	3	6.5	86.4
	Selibate®CS	900	30	30	5.1	67.8
2012	Neburel®-Z	100	3	3	7.9	105
	Neburel®-M	100	3	3	6.5	86.4
	Untreated	100	-	-	-	-
	Selibate®CS	700	30	30	5.1	67.8
2013	Saturel®M-5	100	5	60	6.3	83.4
	Saturel®M-10	100	10	60	6.3	83.4
	Selibate®CS	900	30	30	5.1	67.8

[a] Mating disruption treatments with passive hand-applied dispensers (Saturel® and Selibate®CS) and aerosol devices (Neburel®).

[b] Total pheromone (three-component blend or major component) emitted during the studied periods, from June to mid-September.

[c] Mean release rate of each type of dispenser calculated by solvent extraction and GC analysis of residual pheromone at different aging times for Saturel® and Selibate® dispensers, and the gravimetric method for Neburel® devices.

Figure captions

Fig. 1 Saturel® dispensers employed in Experiment 3 (2013): (A) 6 Saturel®-M: 3 units of mesoporous dispensers on each side of the blister; (B) 12 Saturel®-M: 6 units of mesoporous dispensers on each side of the blister.

Fig. 2 Arrangement of mating disruption treatments in the different field experiments. (A) Experiment 1 (2011): Neburel®-Z and Neburel®-M (3 devices/ha), Selibate®CS and Saturel® (30 points/ha). (B) Experiment 2 (2012): Selibate®CS (30 points/ha), Neburel®-Z and Neburel®-M (3 devices/ha), and untreated area. (C) Experiment 3 (2013): Selibate®CS (30 points/ha), Saturel®-M (5 points/ha) and Saturel®-M (10 points/ha).

Fig. 3 Results of Experiment 1 (2011): (A) Captures of *Chilo suppressalis* (mean ± SEM males/trap/day) recorded in the monitoring traps located in each pheromone treated area; (B) crop damage (mean percentage of infested plants ± SEM) resulting from the different mating disruption treatments (bars labelled with the same letter are not significantly different; Tukey HSD test).

Fig. 4 Results of Experiment 2 (2012): (A) Captures of *Chilo suppressalis* (mean ± SEM males/trap/day) recorded in the monitoring traps located in each pheromone treated area; (B) crop damage (mean percentage of infested plants ± SEM) resulting from the different mating disruption treatments (bars labelled with the same letter are not significantly different; Tukey HSD test).

Fig. 5 Results of Experiment 3 (2013): (A) Captures of *Chilo suppressalis* (mean ± SEM males/trap/day) recorded in the monitoring traps located in each pheromone treated area; (B) crop damage (mean percentage of infested plants ± SEM) resulting from the different mating disruption treatments (bars labelled with the same letter are not significantly different; Tukey HSD test).