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Spodoptera littoralis male capture suppression in processing spinach using two kinds of synthetic sex-pheromone dispensers

Alberto Lanzoni[1], Giovanni G. Bazzocchi[1], Franca Reggiori[2], Franco Rama[2], Luigi Sannino[3], Stefano Maini[1], Giovanni Burgio[1]
[1]*Dipartimento di Scienze Agrarie - Entomologia, Università di Bologna, Italy*
[2]*Isagro Ricerca s.r.l., Novara, Italy*
[3]*Unità di Ricerca per le Colture Alternative al Tabacco (CRA-CAT), Scafati, Italy*

Abstract

The efficacy of mating disruption was evaluated against *Spodoptera littoralis* (Boisduval) infesting processing spinach (*Spinacia oleracea* L.) in a two year field study. Two different kinds of pheromone dispenser were utilized during the study, including Ecodian® pheromone dispenser and a new thread dispenser. Pheromone treatments were effective in controlling *S. littoralis* populations, with a male capture reduction ranging from 94.6 to 98.9%. The drastically reduced adult male catches resulted in a lowering of larval populations in comparison with untreated plots. Pheromone treatments, in some cases, did not maintain the infestation below the very restrictive economic threshold required by the processing industry. Manual application of Ecodian® pheromone dispenser was a particularly time-consuming technique and not suitable for field vegetable crops. The thread application resulted in an easier solution, even if a mechanized field installation could further improve the method.

Key words: *Spodoptera littoralis*, mating disruption, thread dispenser, pheromone, processing spinach.

Introduction

Cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera Noctuidae), is recorded in Africa, Southern Europe and Asia Minor and it is considered a noxious pest of many crops within Mediterranean area (Carter, 1984; Pineda *et al*., 2007). It is one of the most commonly intercepted species in Europe, for example on imported ornamentals. It has been found but has not yet established in western and northern Europe (Denmark, Finland, France, Germany, Netherlands, England) and it is a potentially serious pest of glasshouse crops in northern Europe (DAISIE, 2006).

This polyphagous insect pest is one of the most dangerous moths in central and southern Italy (Sannino, 2003). *S. littoralis* attacks most vegetable crops, including tomato, pepper, eggplant, lettuce, artichoke, strawberry, asparagus, but it may also causes damage on ornamental plants and herbs (Sannino, 2003). Recently, it has become a key pest on spinach (Lanzoni and Burgio, 2010). In particular, the protection of processing spinach shows many problems related to the very low economic threshold of pests: the commercial damage is indirectly caused by the presence of larvae, which can make the product unmarketable (Lanzoni and Burgio, 2010). This characteristic has important implications in the application of control methods in a context of low input, sustainable agriculture: treatments with conventional insecticides, in fact, may lead to technical problems and adverse effects, such as the selection of resistant insect populations or the presence of residues at harvest exceeding the legal limits.

Mating disruption is a direct control tactic of insects based on pheromone use (Suckling and Karg, 2000). This technique seems particularly suitable for sustainable agriculture because it minimizes the deleterious effects on non-target fauna, including beneficial arthropods, leading to a reduction or elimination of insecticide treatments. In addition, since mating disruption may be combined with low impact microbial control, it could be potentially applicable to control pests in the organically managed agroecosystems. Mating disruption was tested on *Spodoptera* spp. infesting vegetable crops and cotton (Kehat *et al*., 1983; de Souza *et al*., 1993; Kerns, 2000), showing to be a potential method to control pest populations.

The general aim of this study was to evaluate the efficacy of mating disruption against *S. littoralis* infesting spinach. This technique seems to be particularly suitable for processing spinach, because it can minimize the amount of insecticide residues in a crop characterized by a short cycle, improving the ecological impact of the cultivation technique. A specific objective was to assess a new kind of pheromone-impregnated-thread dispenser (Rama *et al*., 2009). This biodegradable, low-dosage, slow-release pheromone dispenser has been tested in glasshouse and open field on cyclamen and herbs, proving to be effective in decreasing *S. littoralis* males trap catches and damage on leaves (Rama *et al*., 2011). It can be easily used for a wide range of crops, such as vegetable or flowers that, unlike orchards, where the dispensers are applied directly to the branches of the trees, are usually lacking suitable supports for an adequate number of uniformly distributed dispensers. This pheromone-impregnated-thread dispenser could be a solution for a crop like spinach, cultivated on large areas, minimizing the costs of distribution of the pheromones.

Materials and methods

Experimental sites

A two year study (2006 and 2007) was conducted on processing spinach cultivated in the area of Latina province, central Italy (41°29'58.73"N, 12°57'50.67"E). Both the years, the crop was sown in September and harvested in November. A flat-leafed spinach variety was sown, with a distance of 0.15 m among rows.

In the 2006 trial site, a 1 ha pheromone-treated plot and a 1 ha control plot which did not receive any insecticide sprays (both approximately 100 × 100 m) were delimited in two different spinach fields (3.5 and 2.5 ha respectively).

In 2007 the test site was located in the same area as the previous year, and consisted of two 1 ha pheromone-treated plots and two 1 ha control plots which did not receive any insecticide sprays (each 100 × 100 m) within three different spinach fields (3.5, 5, and 3.7 ha respectively). In both years, the control plots were located upwind, approximately 600-1000 m away from the treated plots to avoid pheromone drift into the untreated plots. Pheromone-treated and control plots were always bordering spinach fields in which conventional control measures were applied.

Pheromone treatments

Two different kind of pheromone dispenser were utilized during the study. In 2006, Ecodian® dispenser hooks (Isagro Italia, Italy), each containing approximately 12.5 mg of a 95:5 pheromone mixture of (Z,E)-9,11-14:Ac and (Z,E)-9,12-14:Ac, were used in the trial. Each dispenser consisted of biodegradable materials (Mater-Bi®, Novamont, Novara, Italy) that were shaped into a hook. The trial was laid out in 1 day on 27 September 2006, two weeks after sowing. Dispensers were each attached, using a metal wire, to 1 m split bamboo canes in such a way that the dispensers were 0.8 m above the crop canopy, and uniformly distributed by hand, in the 1 ha trial plot at a rate of 600 dispensers/ha (7.5 g a.i. per ha). Before harvest on 31 October 2006 all canes and dispensers were removed from the field.

In 2007, a novel thread dispenser called Pheromone-Impregnated-Thread (PIT) dispenser (Rama *et al*., 2011) was tested. PIT consists of a continuous thread in which a paper inner core impregnated with a 95:5 blend of (Z,E)-9,11-14:Ac and (Z,E)-9,12-14:Ac is covered by an outer layer of biodegradable material (Mater-Bi®). Each thread dispenser is about 100 m long. The diameter of PIT dispenser is about 3 mm and the average content of pheromone blend was assessed to be 20.3 mg/m. Trials were each laid out in 1 day on 19 September 2007 and on 20 September 2007 both a week after sowing. Thread dispensers were placed in rows of 100 m in each of the two 1 ha trial plots, and to prevent the pheromone thread dispenser from coming into contact with the ground or the plant canopy, it was attached to metal rods placed every 15 m on the row; in such a way the thread dispenser was 0.8-1.0 m above the crop canopy. The distance between two treated rows was 20 m, leading to an application rate of 500 m of thread dispenser per ha (10.2 g a.i. per ha). Before harvest on 31 October and 7 November 2007 all rods and thread dispensers were removed from the fields.

Male capture suppression evaluation

Pheromone traps were used to assess if male *S. littoralis* moths could locate a pheromone point source in the pheromone-treated and in the control plots as well as in the spinach fields outside the pheromone-treated plots. Unitrap® green/yellow/transparent bucket traps (Pherobank, Plant Research International, Wageningen, NL) were used to catch the male moths. Four bucket traps were placed in each of the pheromone-treated and control plots. Traps were baited with rubber *S. littoralis* pheromone lures (Isagro Italia, Italy). Baited traps were hung at about 80-100 cm above the ground and the baits renewed at intervals of 20 days throughout the sampling period. Each trap had an insect killing strip (a.i. 15% diazinon) at the bottom of the trap. Moths were collected from the bucket trap every 5-8 days.

In 2006 trial, besides the four traps placed inside the pheromone-treated plot, other four bucket traps were placed in the spinach field, at 31 m from the downwind edge of the pheromone-treated plot. In 2007 trials, besides the four traps placed inside each of the two pheromone-treated plots, a grid of 16 bucket traps was placed to cover all the area of each of the two spinach fields where the pheromone-treated plots were placed. In these fields all the traps were geo-referenced using a handheld Magellan SporTrak Map® GPS unit. All traps were placed on 27 September in the 2006 trial and on 19 and 20 September in the 2007 trials, at the beginning of the second peak of flight of *S. littoralis* adults in the area of the study (Sannino, 2003).

Larval population estimation

S. littoralis larval populations were estimated within each of the pheromone-treated and control plots using a hand-held vacuum suction device (modified, reversed Stihl BG75 leaf blower), without damaging spinach plants. Each vacuum sample consisted of 20 one-second suction samples taken while moving up and down the spinach rows in a 2 × 2 m spinach area. The central part of each 1 ha tested plots was sampled at a rate of 8 suction areas randomly selected (4 in 2006) on a weekly basis.

Pheromone release rate

The release rate of the pheromone from the thread dispenser was determined under field conditions in 2007 on field 1. The first samples were collected immediately after field application; subsequently the samples were collected on a weekly basis until the end of the trial. Each sample consisted of about 15 cm of PIT dispenser obtained by cutting the thread dispensers in the field at one of their end. Samples were then stored at −20 °C until the end of the trial when they were analyzed for residual pheromone, as follows: 10 cm of thread were cut into pieces and left to soak overnight in 20 ml of tetrahydrofurane containing 0.2 mg/ml of n-hexadecanol as internal standard. The samples were centrifuged at 4500 rpm for 15 minutes and the supernatant was analyzed by gas chromatography.

Data analysis

The evaluation of efficacy of pheromone treatment in treated *vs*. control plots was performed under the assumption of "observational survey" (Schwarz, 1998). By this approach, different plots were selected and area samples were located within each plot; the response variables were the amount of larval and adult infestations in each plot.

The assessment of pheromone treatment efficacy was carried out by means of different variables: i) comparison of the male catches in the pheromone treated and untreated plots, by Kruskal-Wallis test ($P < 0.05$); disruption efficacy was also evaluated as percentage of catch reduction; ii) comparison of larval infestation in the pheromone treated and untreated plots, by Kruskal-Wallis test ($P < 0.05$); iii) comparison of the larval infestation in pheromone treated and untreated plots with an economic threshold for processing spinach, estimated by sampling the larval infestation (number of larvae per 4 m^2) in standard insecticide-treated spinach fields (Anonymous, 2003) at harvest, using the hand-held vacuum suction device (see section 2.4 of materials and methods). The economic threshold (ET) was calculated as: ET = x + 75th percentile, where x is the median. The comparison of the larval infestation of each treatment with the ET was performed by the "confidence interval method", suggested and described in Berthouex and Brown (2002), using a non parametric approach based on medians and percentiles; the use of non-parametric statistics was necessary because of deviation from normality of field data, which showed strong asymmetrical distribution (Zar, 1984). The STATISTICA software for Windows StatSoft Inc. (2011) was used for statistical analysis.

Efficacy of pheromone treatments was also evaluated by mapping male catches per trap in pheromone treated area, using geostatistics (Rossi *et al*., 1993; Journel and Huijbregts, 2003; De Luigi *et al*., 2011). Semi-variograms of the total catches were calculated. The best semi-variogram model was selected by interpreting the model outputs (Rossi *et al*., 1993; Toepfer *et al*., 2007). Ordinary kriging was used as interpolation method and maps obtained were validated by cross-validation analysis, comparing the predicted and observed values by means of linear correlation analysis. Only maps which were significant by cross-validation analysis were presented. Geostatistic data were analyzed using ArcGIS™ (ESRI, Redlands, CA, USA), with the geostatistic ArcMap™ (ESRI, Redlands, CA, USA) extension.

Results

In both years the number of male moths caught in bucket traps in pheromone-treated plots was significantly lower compared to control plots on all sampling date and at each test site. Disruption efficacy, evaluated as percentage of catches reduction in treated against untreated plots, ranged from 92.3 to 100% in 2006 and from 97.4 to 99.4% in field 1, and from 91.6 to 98.1 in field 2 in 2007 (tables 1 and 2). In the 2006 trial, in the

Table 1. Mean number (± SE) of *S. littoralis* male trapped per night in pheromone traps and percentage of disruption efficacy in pheromone-treated, 31 m away, and control treatments in 2006 trial.

Date	Nights	Treatment	Mean moths per trap per night ± SE[a]	Disruption efficacy (%)	Median (25th and 75th percentiles)
12 Oct	8	Pheromone	0.03 ± 0.03a[c]	93.8	0.0 (0.0 - 0.063)
		31 m away[b]	0.19 ± 0.15ab	62.5	0.06 (0.0 - 0.38)
		Control	0.50 ± 0.11b		0.50 (0.31 - 0.69)
		H (*df*) *P*	6.281 (2, 12) 0.0433		
17 Oct	5	Pheromone	0.00 ± 0.00a	100	0 (0 - 0)
		31 m away	0.20 ± 0.12ab	75.0	0.20 (0.0 - 0.40)
		Control	0.80 ± 0.20b		1,0 (0.60 - 1.0)
		H (*df*) *P*	7.691 (2, 12) 0.0214		
24 Oct	7	Pheromone	0.04 ± 0.04a	97.7	0.0 (0.0 - 0.071)
		31 m away	0.39 ± 0.04ab	74.4	0.43 (0.36 - 0.43)
		Control	1.54 ± 0.16b		1.57 (1.29 - 1.79)
		H (*df*) *P*	10.130 (2, 12) 0.0063		
30 Oct	6	Pheromone	0.00 ± 0.00a	100	0 (0 - 0)
		31 m away	0.25 ± 0.14ab	92.3	0.17 (0.08 - 0.42)
		Control	3.25 ± 0.84b		3.75 (2.17 - 4.33)
		H (*df*) *P*	9.464 (2, 12) 0.0088		
Total (12 Oct - 30 Oct)		Pheromone	0.07 ± 0.04a	98.9	0.063 (0.0 - 0.134)
		31 m away	1.03 ± 0.28ab	83.1	1.04 (0.57 - 1.49)
		Control	6.09 ± 1.22b		6.82 (4.61 - 7.56)
		H (*df*) *P*	9.881 (2, 12) 0.0072		

[a] Data were analyzed using Kruskal-Wallis test. Means followed by the same letter are not significantly different at $P < 0.05$ by mean ranks multiple comparisons test; [b] Pheromone traps placed 31 m away from the downwind edge of the pheromone-treated plot; [c] $P = 0.0558$.

Table 2. Mean number (± SE) of *S. littoralis* male trapped per night in pheromone traps and percentage of disruption efficacy in pheromone-treated and control spinach plots in 2007 trials.

Date	Nights	Treatment	Mean moths per trap per night ± SE[a]	Disruption efficacy (%)	Median (25th and 75th percentiles)
04 Oct	7	Pheromone field 1	0.39 ± 0.11	98.7	0.29 (0.29 - 0.50)
		Control field 1	29.04 ± 7.24		28.79 (17.14 - 40.93)
		H (*df*) *P*	5.600 (1, 8) 0.0180		
11 Oct	7	Pheromone field 1	0.21 ± 0.04	97.7	0.21 (0.14 - 0.29)
		Control field 1	9.36 ± 2.38		10.50 (5.71 - 13.0)
		H (*df*) *P*	5.463 (1, 8) 0.0194		
17 Oct	6	Pheromone field 1	0.17 ± 0.00	97.4	0.17 (0.17 - 0.17)
		Control field 1	6.29 ± 0.98		6.67 (4.83 - 7.75)
		H (*df*) *P*	6.054 (1, 8) 0.0139		
24 Oct	7	Pheromone field 1	0.04 ± 0.04	98.8	0.0 (0.0 - 0.07)
		Control field 1	3.00 ± 0.53		2.57 (2.43 - 3.57)
		H (*df*) *P*	5.671 (1, 8) 0.0172		
30 Oct	6	Pheromone field 1	0.08 ± 0.05	98.2	0.08 (0.0 - 0.17)
		Control field 1	4.58 ± 0.92		4.42 (3.0 - 6.17)
		H (*df*) *P*	5.531 (1, 8) 0.0187		
06 Nov	7	Pheromone field 1	0.11 ± 0.07	99.4	0.07 (0.0 - 0.21)
		Control field 1	16.96 ± 2.47		16.71 (13.43 - 20.50)
		H (*df*) *P*	5.398 (1, 8) 0.0202		
Total (04 Oct - 06 Nov)		Pheromone field 1	0.89 ± 0.15	98.3	0.75 (0.74 - 1.05)
		Control field 1	52.27 ± 11.57		53.64 (33.26 - 71.27)
		H (*df*) *P*	5.398 (1, 8) 0.0202		
04 Oct	7	Pheromone field 2	0.96 ± 0.31	93.0	1.14 (0.50 - 1.43)
		Control field 2	13.79 ± 1.77		12.57 (11.36 - 16.21)
		H (*df*) *P*	5.398 (1, 8) 0.0202		
11 Oct	7	Pheromone field 2	0.29 ± 0.06	91.6	0.29 (0.21 - 0.36)
		Control field 2	3.39 ± 0.99		3.36 (2.14 - 4.64)
		H (*df*) *P*	5.398 (1, 8) 0.0202		
17 Oct	6	Pheromone field 2	0.13 ± 0.08	98.1	0.08 (0.0 - 0.25)
		Control field 2	6.75 ± 1.23		5.92 (5.25 - 8.25)
		H (*df*) *P*	5.398 (1, 8) 0.0202		
24 Oct	7	Pheromone field 2	0.18 ± 0.04	94.3	0.14 (0.14 - 0.21)
		Control field 2	3.14 ± 0.83		2.79 (2.0 - 4.29)
		H (*df*) *P*	5.60 (1, 8) 0.0180		
30 Oct	6	Pheromone field 2	0.17 ± 0.07	96.4	0.17 (0.08 - 0.25)
		Control field 2	4.58 ± 0.92		4.42 (3.0 - 6.17)
		H (*df*) *P*	5.463 (1, 8) 0.0194		
Total (04 Oct - 30 Oct)		Pheromone field 2	1.72 ± 0.38	94.6	2.02 (1.31 - 2.13)
		Control field 2	31.65 ± 3.99		28.54 (26.99 - 36.32)
		H (*df*) *P*	5.398 (1, 8) 0.0202		

[a] Data were analyzed using Kruskal-Wallis test.

control plot, *S. littoralis* male moth catches were very low, and peaked at 3.25 moths/trap/night at the end of the study (30 October). A total of 6.09 moths per trap per night was observed in control plot during trial conduct (33 days) indicating that pest pressure at trial site was moderate (table 1). On the contrary, in 2007, a total of 52.27 and 31.65 moths per trap per night was observed in control plot in field 1 and 2 respectively, throughout the trial periods (48 and 40 days), indicating a higher level of *S. littoralis* moth activity. The main peak of catches was observed at the beginning of the trial on 4 October, with 29.04 and 13.79 moths per trap per night in field 1 and 2 respectively (table 2). A second peak of adult moth activity was recorded toward the end of the trials on 6 November in field 1 (16.96 moths per trap per night).

Further, in 2006 trial, male moth counts from traps placed 31 m away from the downwind edge of the pheromone-treated plot were lower than that from traps placed in the control plot, with disruption efficacy ranging from 62.5 to 92.3%, and just slightly higher than from traps placed in the inner of pheromone-treated plot. However, these differences were not significant (table 1).

The trap catch reduction within pheromone-treated field can be visualized from the geostatistic surface maps (figure 1). The gaps of catches within the square areas in the maps, which represent the treated plots,

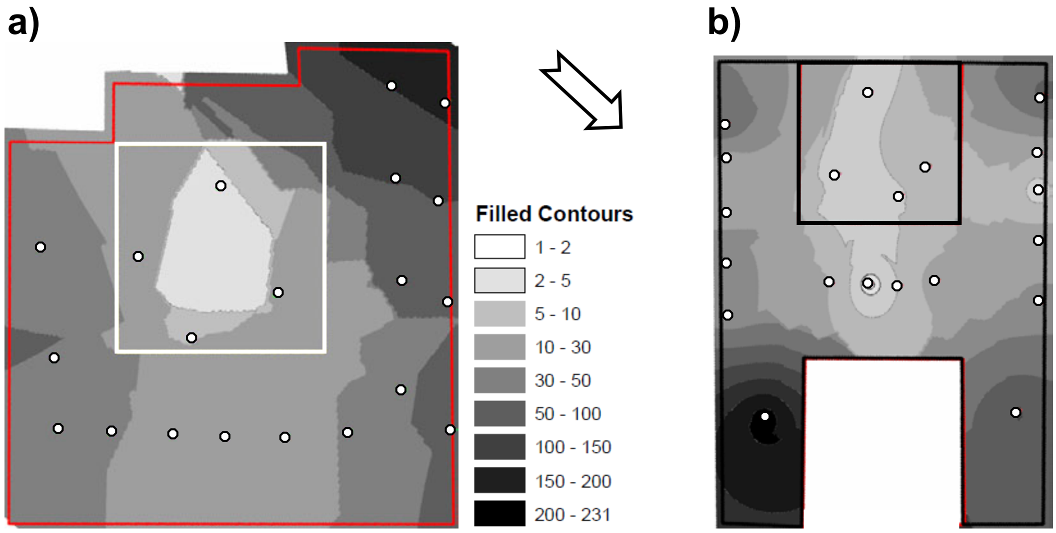

Figure 1. Spatial distribution of total male catches per trap in pheromone treated spinach fields over 5 consecutive weeks in pheromone field 1 (a) and over 4 in pheromone field 2 (b) in 2007 trials. The 1-ha pheromone treated plot is indicated by the square (in white in field 1 and in black in field 2). Dots represent traps position. Arrow represents dominant wind direction.

demonstrated the effectiveness of the catches reduction due to the technique. The maps showed also the pheromone drift from the pheromone-treated plots, showing a partial efficacy of the disruption technique outside the pheromone-treated area. The patches of the catches, visualized by the darker filled contours, indicated the areas of the fields in which male disruption was not effective.

In 2006, a significant reduction of *S. littoralis* larvae in the pheromone-treated plot, respect to the control, was detected in all the sampling dates and in total, despite *S. littoralis* population was very low, reaching a peak of 0.68 larvae/4 m^2 in the control plot (table 3). Moreover, the larval infestation sampled in pheromone-treated plot one day before harvest (30 October) was as low as the ET calculated in the insecticide-treated spinach fields (figure 2).

In 2007, only in the sampling carried out on the 6 of November in field 1 the pheromone-treated plot contained significantly fewer *S. littoralis* larvae than the control plot, when infestation in the latter plot peaked at 19.75 larvae/4 m^2 (table 4). In all the other sampling dates, both in field 1 and 2, when larval populations were low, a significant reduction in *S. littoralis* larvae was not observed (table 4). This lack of significant effect at low larval infestation is not in agreement with what was found in 2006. This was probably due to the different distribution of the larval population, as showed by the median and percentile values (tables 3 and 4). In the second year, pheromone treated plots showed larval infestation levels higher than the economic threshold calculated in insecticide-treated plots (figure 2). Larval infestation in control field 2 was drastically lower than that found in control field 1; this difference was due to the early harvest in field 2, which minimized the larval infestation in this plot.

The relationship between residual pheromone and time in the thread dispenser showed a classical exponential decay law ($Y = 100.3^{*}\ e^{-0.043^{*}x}$; $R^2 = 0.99$, $P < 0.001$) (figure 3). The percentage of pheromone remaining in the thread dispenser after 15 days of field aging was 50%,

Table 3. Mean number (± SE) of *S. littoralis* larvae sampled in a 2 × 2 m spinach area in pheromone-treated and control spinach plots in 2006 trial.

Date	Treatment	Mean larvae ± SE[a]	Median (25th and 75th percentiles)
17 Oct	Pheromone	0.00 ± 0.00	0 (0 - 0)
	Control	0.68 ± 0.33	0.51 (0.17 - 1.19)
	H (*df*) *P*	6.137 (1, 8) 0.0132	
24 Oct	Pheromone	0.00 ± 0.00	0 (0 - 0)
	Control	0.47 ± 0.08	0.43 (0.34 - 0.60)
	H (*df*) *P*	6.137 (1, 8) 0.0132	
30 Oct	Pheromone	0.00 ± 0.00	0 (0 - 0)
	Control	0.30 ± 0.09	0.26 (0.17 - 0.43)
	H (*df*) *P*	6.137 (1, 8) 0.0132	
Total (17 Oct - 30 Oct)	Pheromone	0.00 ± 0.00	0 (0 - 0)
	Control	1.45 ± 0.21	1.36 (1.19 - 1.70)
	H (*df*) *P*	6.054 (1, 8) 0.0139	

[a] Data were analyzed using Kruskal-Wallis test.

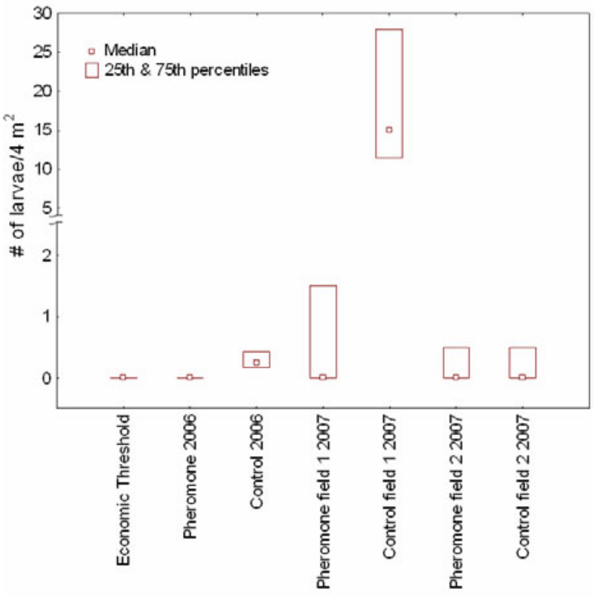

Figure 2. Comparison of the number (Median and 25th and 75th percentiles) of *S. littoralis* larvae sampled at harvest in a 2 × 2 m spinach area in pheromone-treated and control plots in 2006 and 2007 trials with the estimated Economic Threshold.

while at the end of the spinach cropping cycle (48th day) the percentage resulted to be 9%. The average release rate during the first 15 days was 0.66 mg/m per day, which decreased to 0.26 mg/m per day in the remaining period (mean value between days 16 to 49). Since 500 m/ha of thread dispenser was used, and assuming a release of 0.26 mg/m per day, the release rate tested was estimated as 5.5 mg/h per ha.

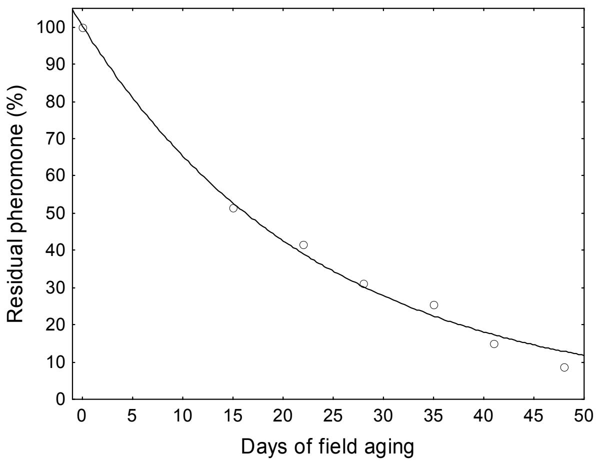

Figure 3. Percentages of pheromone remaining in Pheromone-Impregnated-Thread dispenser utilized in 2007.

Discussion

Overall, pheromone treatments were shown to be effective in controlling *S. littoralis* populations. As a matter of fact, this technique determined a drastic reduction in adult male catches, thus resulting in a lowering of larval populations in comparison with untreated plots.

Our findings indicated that both the Ecodian® dispensers tested in 2006 and the thread dispenser tested in 2007 effectively disrupted *S. littoralis* male orientation toward pheromone sources, likely resulting in an almost complete disruption of mating. However some mating could have taken place in the pheromone-treated plots, since a small number of male were captured in the traps inside these plots.

Table 4. Mean number (± SE) of *S. littoralis* larvae sampled in a 2 × 2 m spinach area in pheromone-treated and control spinach plots in 2007 trials.

Date	Treatment	Mean larvae ± SE[a]	Median (25th and 75th percentiles)
	Pheromone field 1	0.00 ± 0.00	0 (0 - 0)
11 Oct	Control field 1	0.25 ± 0.16	0.0 (0.0 - 0.50)
	H (*df*) *P*	2.143 (1, 16) 0.1432	
	Pheromone field 1	0.13 ± 0.13	0 (0 - 0)
17 Oct	Control field 1	0.13 ± 0.13	0 (0 - 0)
	H (*df*) *P*	0 (1, 16) 1.0	
	Pheromone field 1	0.50 ± 0.50	0 (0 - 0)
30 Oct	Control field 1	0.75 ± 0.31	0.50 (0.0 - 1.50)
	H (*df*) *P*	1.638 (1, 16) 0.2007	
	Pheromone field 1	1.63 ± 1.25	0.0 (0.0 - 1.50)
06 Nov	Control field 1	19.75 ± 6.91	15.0 (11.50 - 28.0)
	H (*df*) *P*	7.395 (1, 12) 0.0065	
	Pheromone field 2	0.38 ± 0.18	0.0 (0.0 - 1.0)
11 Oct	Control field 2	0.38 ± 0.38	0 (0 - 0)
	H (*df*) *P*	0.813 (1, 16) 0.3674	
	Pheromone field 2	0.75 ± 0.31	0.50 (0.0 - 1.50)
17 Oct	Control field 2	0.25 ± 0.25	0 (0 - 0)
	H (*df*) *P*	1.995 (1, 16) 0.1579	
	Pheromone field 2	0.38 ± 0.26	0.0 (0.0 - 0.50)
30 Oct	Control field 2	0.25 ± 0.16	0.0 (0.0 - 0.50)
	H (*df*) *P*	0.019 (1, 8) 0.8897	

[a] Data were analyzed using Kruskal-Wallis test.

In 2006, the larval infestation was completely eliminated in the pheromone-treated plot, while in 2007 the reduction in *S. littoralis* larval populations early in the cropping cycle was not consistent probably because of the higher level of *S. littoralis* activity registered at the beginning of the study as well as greater data variability. Moreover, mating had certainly occurred in the spinach fields were the 1-ha pheromone-treated plots have been delimited and then gravid female could have moved inside the treated plots to lay eggs. Geostatistics can be a useful tool to analyze the efficacy of the pheromone-treated plots, through representation of spatial pattern of catches. Gaps and patches of catches can be interpreted as areas in which catches reduction is optimal or ineffective, respectively. Kerns (2000), studying *Spodoptera exigua* (Hubner), and de Souza *et al*. (1993), both underlined the importance of the pattern of dispenser distribution over a much greater area outside the cropped field, in order to let mating disruption efficacious. The geostatistical analyses of trap catches reported in our study seem to sustain this interpretation and allowed visualization of the magnitude of the phenomenon. Actually, the pheromone point-source location was effectively disrupted only in the central part of the 1-ha pheromone-treated plots. This underlines the importance of extending the pheromone-treated area outside the cropped area, as reported by Kerns (2000) and seen the better result obtained by Wakamura and Takai (1995) with *S. exigua*.

In the literature, the pheromone doses used to disrupt *S. littoralis* males showed a consistent variation, ranging from 0.39 to 60 g/ha (Kehat *et al*., 1983; de Souza *et al*., 1993, Rama *et al*., 2011). In our study, the use of 7.5 g/ha in 2006 resulted in a 98.9% male capture reduction. In 2007 the dose of 10.2 g/ha was used, with an efficacy of 98.3 to 94.6% in field 1 and 2 respectively. Pheromone release rates substantially decreased after 15 days of field aging. However, after that, the rate of pheromone emission (5.5 mg/h per ha) was still enough to sustain good mating disruption in the field. Kehat *et al*. (1979) report effective communication disruption on *S. littoralis* with release rates ranging between 2.0 and 4.0 mg/h per ha. Release rate of 1.0 mg/h per ha resulted in decreased disruption efficiency and when the pheromone emitted was increased to 12.2 mg/h per ha, disruption was not improved (Kehat *et al*., 1979). These rates are consistent with the release rate found to be effective in our study, even if in the first 15 days of the trial also a release rate of 13.7 mg/h per ha sustained a 98.7% disruption efficacy.

It is remarkable that male capture reduction in our study persisted also with a 15% of the initial dose of pheromone, corresponding to a residual of 1.5 g/ha. This was the same pheromone amount required to effectively disrupt communication of *S. littoralis* in cotton for about 4 weeks (Kehat *et al*., 1983). The pheromone release rate seems to provide efficacy of the method through the whole spinach cultivation period. Moreover, the low residual amount of pheromone in the thread dispenser at the end of the cultivation period (9%) suggests that the quantity of pheromone needed and the related costs could be minimized by using this type of dispenser.

Nevertheless, our results indicated that mating disruption failed, in some cases, to control the key pest below the very low economic threshold imposed by the spinach processing industry. Studies are needed to evaluate the potential of the mating disruption approach when combined with other indirect and direct low impact strategies (i.e. integrated pest management) for key pests due to the very restrictive threshold. In this contest, the use of biopesticides as *B. thuringiensis* or nucleopolyhedroviruses (NPV) could represent an integration (Masetti *et al*., 2008). Moreover, the use of biocontrol agents could be a potential strategy to reduce pest resistance caused by intensive use of chemical insecticides and to manage restrictions of current insecticides.

The manually application of the dispensers in 2006, was a particularly time-consuming technique and not suitable with a commercial use. The thread application resulted in an easier solution, even if a mechanized field installation could further improve the device application method.

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Authors' addresses: Giovanni BURGIO (corresponding author, giovanni.burgio@unibo.it), Alberto LANZONI, Giovanni G. BAZZOCCHI, Stefano MAINI, DipSA - Entomologia, *Alma Mater Studiorum* Università di Bologna, viale G. Fanin, 42, 40127 Bologna, Italy; Franca REGGIORI, Franco RAMA, Isagro Ricerca s.r.l., via G. Fauser 4, 28100 Novara, Italy; Luigi SANNINO, Unità di Ricerca per le Colture Alternative al Tabacco (CRA-CAT), via P. Vitiello 106, 84018 Scafati (SA), Italy.

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