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Ostrinia nubilalis in northern Italy: egg parasitoids and control trials on three sweet pepper landraces in open field cultivations

Giuseppe CAMERINI[1], Monica MASANTA[2], Stefano MAINI[3]
[1]*Istituto di Istruzione Superiore "Taramelli-Foscolo", Pavia, Italy*
[2]*Istituto di Istruzione Superiore "A. Maserati", Voghera, Italy*
[3]*Dipartimento di Scienze e Tecnologie Agro-Alimentari (DISTAL), Università di Bologna, Italy*

Abstract

The European corn borer (ECB) *Ostrinia nubilalis* Hubner (Lepidoptera Crambidae) is an important pest of sweet pepper (*Capsicum annuum* L.). This research was aimed at developing a strategy for controlling ECB by integrating the natural action of the egg parasitoids and the application of the microbial insecticide *Bacillus thuringiensis* subsp. *kurtstaki* (*Btk*). Data were gathered from experimental plots, which were studied in five growing seasons in Pavia Province (northern Italy). In the first two seasons, three selected pepper landraces ('Asti', 'Cuneo', 'Voghera') were compared to assess their host plant resistance. In addition, data on both oviposition by ECB and egg mass parasitism by *Trichogramma* species (Hymenoptera Trichogrammatidae) were collected. The combined effect of *Trichogramma brassicae* Bezdenko and *Btk* sprays was analysed in comparison with conventional deltamethrin applications. Pepper landraces were differently vulnerable to ECB attacks: 'Voghera' sweet pepper plants proved to be less damaged by larvae in comparison to other landraces. ECB oviposition mainly occurred late in the summer, starting in August. ECB infestations can be related to the spillover of ECB adults from maize fields. Parasitism by *T. brassicae* was regularly observed. It tended to increase following a trend of greater ECB egg masses oviposition. Another egg parasitoid, *Trichogramma agrotidis* Voegele et Pintureau was recorded for the first time on ECB eggs in Italy. Natural ECB control from wild *T. brassicae* and *T. agrotidis*, integrated with *Btk*, can support a synthetic chemical pesticide free strategy for pepper crop protection. Damage to pepper production was kept on average below 5%. Treatments with *Btk* at 6-day intervals, as replacement to deltamethrin applications, allowed an enhancement of agroecosystem sustainability by favouring ECB natural biocontrol.

Key words: biological control, *Trichogramma brassicae*, *Trichogramma agrotidis*, ECB egg masses, parasitism, *Bacillus thuringiensis* subsp. *kurstaki*.

Introduction

Sweet pepper (*Capsicum annuum* L.) is a horticultural crop cultivated all over the world (Lin *et al*., 2013).

Several pests that can significantly damage this crop have been recorded. In Europe and North America severe damage can be caused by European corn borer (ECB) *Ostrinia nubilalis* Hubner (Lepidoptera Crambidae) (Maini and Burgio, 1994; Burgio and Maini, 1995).

The economic importance of ECB on pepper is evidenced by the extensive effort of researchers aimed at finding effective strategies based on integrated management protocols (Elliot *et al*.,1978; 1982; Burbutis and Koepke, 1981; Burgio and Maini, 1995; Barlow and Kuhar, 2004; Kuhar *et al*., 2004; Kahrer, 2005; Hitchner and Ghidiu, 2006; Chapman *et al*., 2009; Russel and Bessin, 2009; Larue and Welty, 2010; Boisclair *et al*., 2011; Ferguson and MacDonald, 2011; Bickerton *et al*., 2012).

This paper reports the results coming from research on ECB as a pest of sweet pepper in the open field. Three Italian sweet pepper landraces were examined: 'Asti', 'Cuneo' and 'Voghera'. By choice the first two landraces were selected by farmers in the Italian region of Piedmont (western Po river valley) although they are also traditionally cultivated outside the area of origin. The 'Asti' landrace is also called 'quadrato d'Asti'. The 'Cuneo' pepper genome was recently resequenced by Acquadro *et al*. (2020). The 'Voghera' sweet pepper faced the risk of extinction, but, thanks to a rescue project and selection conducted in the province of Pavia (Lombardy, central western Po river valley), has been recovered and it is now cultivated as a cash crop product (Cavagna *et al*., 2012).

The protection of pepper landraces from ECB attacks is usually based on insecticide sprays, or nets as a means of exclusion (Weintraub, 2007). In the Po river valley, ECB is mainly distributed in maize, but other crops can be impacted (Camerini *et al*., 2014; 2018). ECB usually develops two generations of larvae. Two or three flights can be observed. The first flight of adults coming from the overwintering mature larvae is observed from the end of May to the first decade of June, when a maximum of adults usually can be recorded. Then, a second moth flight peak is recorded in the second half of August (Camerini *et al*., 2015). Moreover, in case of special favourable climatic conditions, a third flight producing a partial generation of larvae can be recorded.

Several native natural enemies - mainly insects - are active against ECB (Magagnoli *et al*., 2021). Among them *Trichogramma brassicae* Bezdenko (= *T. maidis* Pintureau et Voegele) (Hymenoptera Trichogrammatidae) and *Lydella thompsoni* Herting (Diptera Tachinidae) are the most effective (Maini *et al*., 1983; Camerini *et al*., 2016; 2018). In the study area the activity of egg parasitoid *T. brassicae* on ECB first egg masses generation tends to be variable year by year (Camerini *et al*., 2016). Parasitism levels of ECB second generation eggs usually are low in July, but tend to rise to relevant values at the end of maize growing season (Camerini et al., 2018; Magagnoli *et al.*, 2021). ECB egg masses laid on sweet pepper plants belong mainly to ECB second and sometimes to third adult flights.

The research was carried out from 2006 to 2010 and consisted of several steps: 1) first evaluation of damage caused by ECB to pepper landraces ('Asti', 'Cuneo', 'Voghera') and assessment of *T. brassicae* role as natural biocontrol agent (years 2006-2007); 2) experimental use of *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) sprays (year 2008); 3) experimental use of *Btk* in comparison to chemical insecticide (deltamethrin) (year 2009); 4) final testing of a biological control protocol based on the integration of *T. brassicae* and *Btk* (year 2010).

A limitation of damage to crop production under the level of 5% was assumed as a target to be achieved at the end of research work.

Materials and methods

Study area

The study area was selected with the aim of testing the high ECB densities impact on sweet pepper production; for this reason, during almost the whole experimental period, sweet pepper plots were located next (<150 m) to maize fields, that work as main reservoir of ECB infestations. Maize is usually sown in March; late planting is uncommon. In Italy GM maize (or Btcorn) is cultivated neither in this area nor in other ones.

The study area lies at 45°05'23"N 9°04'58"E in the municipality of Bastida Pancarana (Pavia Province, northern Italy).

Experimental plots were sown on a loose soil (pH = 7.6; clay 21.8% - silt 61% - sand 17.2%) suitable for horticultural cultivations. Soil was fertilized by applying a mixture of compost and chicken manure, while well water was provided by surface flooding. Plots were not re-sown year by year on the same soil in order to prevent damage caused by parasitic fungi (i.e., *Fusarium* sp.).

In the study area a set of water pan traps (N = 16) were baited with sex pheromone rubber septa (97:3 E/Z 11-tetradecenyl acetate - loading 0.1 mg/lure) and phenylacetaldehyde (PAA 370 mg, stabilized with an UV-screener and an antioxidant, impregnated on a 25 × 25 mm filter paper, 2.7 mm thick). Baits were changed every week. Water pan traps were operative as part of a research on seasonal and diel rhythms of ECB adult activity, which had been carried out from 2008 to 2010 (Camerini *et al.*, 2015). Unlike sex pheromone, PAA can attract both ECB sexes, so that the beginning of females trapping can work as a precise indicator of ECB starting their oviposition on sweet pepper plants (Maini and Burgio, 1984).

In 2009 supplemental observations were made in open field in cooperation with a farm located in Corana (45°04'06"N 8°58'41"E), next to Voghera, in the agricultural district where the same sweet pepper landrace is traditionally grown.

Experiment 1: assessment of damage to crop production and parasitism by *T. brassicae* (seasons 2006-2007)

Sweet pepper plants were sown at the beginning of March. Seedlings were grown in a glasshouse and then transplanted in open field at the beginning of June. Table 1 summarizes data concerning sweet pepper experimental plots devoted to the cultivation of compared landraces ('Asti', 'Cuneo', 'Voghera'). Twelve square-shaped randomized plots were organized according to a common pattern. Every plot was made of 5 rows (6 plants in a row). Plants were tied to a tutor stake in order to prevent any damage coming from the wind.

In every plot a series of 6 plants, 5 of them placed along the diagonal connecting the corners of the plot, were marked and used as samples for monitoring fruit production and damage by ECB. Ripe fruits were weekly collected when their colour turned from green to yellow-pink. After harvest, every fruit was weighed. Fruit skin was examined to record any hole by ECB larvae piercing. Then fruits were cut into halves to find out larvae invading their interior. Damage was calculated as percentage of fruit production wasted by ECB attacks.

Eight plants/landrace (2 plants per plot) were selected and marked as samples for ECB egg masses monitoring, which was carried out every 12 days, from July to the end of September. In response to significant increase of egg masses abundance, the number of sample plants was reduced. Foliage was checked leaf by leaf; both leaf sides were examined. Stems and fruit skin were also checked. Recorded egg masses were removed from the plant and put into a plastic Petri dish (35 mm diameter) and then reared in lab conditions (16:8 L:D - 24-25 °C - 60-70% RH). Fresh ECB egg masses are whitish and this colour does not change until eclosion if no attack by oophagous parasitoids occurs, except for the final "black head" stage. When eggs are attacked by *Trichogramma*, their colour turns from whitish to blackish; in some egg masses both colours can be observed at the same time, when only a part of the egg mass has been parasitized.

Egg masses were daily checked by means of a stereomicroscope in order to estimate the whole number of eggs and, if any, of parasitized ones. When the fate of eggs appeared to be well defined (black head stage or parasitized eggs), egg masses were brought back to experimental plots. Then they were fixed by means of a clip to the plant from which they had been sampled in order to limit possible effects of sampling on pest and parasitoid population dynamics.

Table 1. Data on experimental plots (2006-2007, 2008).

Seasons	2006-2007	2008
Number of plots	12	24
Tested landraces	3	3
Repetition/landrace	4	4
Plants/plot	30	18
Plants/landrace	120	144
Number of plants	360	432
Spacing (cm)	40 × 50	40 × 50

Experiment 2: use of *Btk* (season 2008)

The growing season 2008 was devoted to the trial of *Btk* sprays as an integration of natural contribution given by *T. brassicae* to limiting ECB population. Seeding time of compared landraces, seedling transplantation and spacing were the same as the ones already adopted in 2006-2007 (table 1).

Changes concerned the number of plants a plot (N = 18), that is 3 rows of 6 plants per plot and the number of plots (N = 24 - table1). Twelve plots (4 repetition/landrace), where *Btk* was not sprayed, were used as controls, while twelve plots were devoted to the microbiological insecticide application (4 repetition/landrace).

Bio BIT DF (Valent Biosciences - *B. thuringiensis* subsp. *kurstaki* - serotype 3a-3b) was measured out 600 g/hectare, according to its producer's suggestion referred to application on Solanaceae. In addition to Bio BIT, the adhesive (Etravon - Syngenta) was applied measuring out 0.5 ml/l. Bio BIT was sprayed 7 times every 9 days starting on 24th July, when the first ECB egg masses were recorded.

In every plot two plants (n. 2 and n. 5) of the central row were marked and used as samples for crop production and damage assessment. In such way, a total of 48 plants were periodically checked.

Regular sampling on ECB oviposition and parasitism by *T. brassicae* trends were carried out on plant n. 5 in each plot, according to the methods previously described. At the end of crop seasons the same 24 plants were dug up in order to weigh their stem and leaves and estimate their biomass in order to analyse a potential correlation between foliage plants development and ECB egg masses density.

A sample of maize plant in the field next to the plots was also examined for the assessment of ECB eggs parasitization three times, from the beginning of August to the first half of September, with the aim of assessing ECB parasitized eggs.

Experiment 3: comparison trials *Btk* vs deltamethrin (season 2009)

This growing season was devoted to testing *Btk* microbiological application and comparing it to insecticide spraying. The chemical product (deltamethrin) is the most common used by farmers to protect sweet pepper crops from ECB in the agricultural district where the experimental plots were organized. The biocidal activity of the chemical product (Decis Jet), which was sprayed at 0.8 ml/l concentration (dose 0.8 l/ha), is due to the active ingredient deltamethrin, an insecticide belonging to the pyrethroid family. *Btk* was sprayed every 6 days (starting on 2nd August) while deltamethrin was applied every 10 days.

Plots layout were the ones already adopted in 2008, as well as the number and location of sample plants for ECB egg masses monitoring in every plot. Due to the implementation of deltamethrin application, 36 plots were organized, which means 4 repetitions for every landrace and treatment (control/*Btk*/deltamethrin).

A supplemental trial on *Btk* application was carried out in a farm located in Corana, near Voghera, where the cultivation of the same landraces is a well-established tradition. Trials were exclusively oriented to analyse ECB influence on 'Voghera' landrace. Seedlings were transplanted at the end of June. The field (0.5 ha) was divided into parcels: one for testing the *Btk* sprays, another for evaluating the effects of the deltamethrin sprays, and an untreated control sector including hundred plants.

Eight plants for every trial were marked and periodically monitored in order to record ECB egg masses and damage to crop production.

Experiment 4: final testing of *Btk* (season 2010)

Four years of comparative tests on 'Asti', 'Cuneo' and 'Voghera' sweet pepper landraces were considered enough to assess their susceptibility to ECB attacks, therefore 2010 trials were only devoted to the refinement of the integrated pest management for 'Voghera' sweet peppers (wild *T. brassicae* + *Btk* spray).

The peculiarity of this final test was the distance (> 500 m) of the experimental plots from the nearest maize field. Such a distance was longer than the one separating the experimental plots from the maize field (< 150 m) in previous years.

Btk application frequency (every 6 days) was the same adopted in 2009.

Statistical analysis

Data were analysed by means of Biostat software. Data sets were first examined to analyse their normal distribution according to some specific tests: Kolmogorov-Smirnov/Lilliefor, Shapiro-Wilibuitk, D'Agostino skewness and kurtosis.

χ^2 test and contingency tables were applied to assess whether the frequencies (number of values) in each group of data were significantly different (from each other). Mann-Whitney test was also used for assessing differences between medians.

ANOVA was applied to compare groups of normally distributed data, otherwise Kruskall Wallis test was used.

The association of two ranked variables was assessed by applying the Pearson correlation every time data was not sampled from a normal distribution.

Results and discussion

Experiment 1: assessment of damage to crop production and parasitism by *T. brassicae* (seasons 2006-2007)

The number of ECB egg masses recorded on plants from compared landraces is shown in table 2. The difference was significant. These data suggest a different susceptibility of tested landraces to ECB infestations: the egg masses number on 'Voghera' landrace plants was the smallest both in 2006 and 2007.

Table 2. Number of ECB egg masses laid on sweet pepper plants (P comes from χ^2 test).

Year	2006	2007
'Asti'	68	28
'Cuneo'	74	35
'Voghera'	29	15
P	<0.01	<0.05

Table 3. Damage to crop production ± S.E. (years 2006 and 2007). Letters are referred to pairwise comparison tests (Mann Whitney). Data are compared from the same year. Data from plants attacked by fungi were not computed.

Landrace Year	'Voghera' 2006	'Cuneo' 2006	'Asti' 2006	'Voghera' 2007	'Cuneo' 2007	'Asti' 2007
Sample plants (N) *	23	23	23	23	24	24
Production/plant (g)	1111 ± 96.2	865.7 ± 61.8	1028.2 ± 82	1326.6 ± 91.9	1243.3 ± 109.5	1121.2 ± 125
Damaged production (% weigh)	13.9 (a)	38.9 (b)	40.6 (b)	11.7 (a)	21.9 (ab)	34.8 (b)

Table 3 shows data concerning damage to crop production. The difference among the net production (undamaged fruits) coming from compared landraces was significant in both seasons, 2006 (ANOVA test, $P < 0.01$) and 2007 (ANOVA test, $P < 0.05$). Regarding productions damaged by ECB, the difference was significant in both years (Kruskall Wallis test, $P < 0.05$).

As expected, given the different records of density of egg masses, 'Voghera' resulted to be the landrace least suffering from ECB attacks. On the other hand, the highest loss of production was recorded in 'Asti' plots, even if ECB egg mass density on the plants of this landrace was lower than the one recorded on 'Cuneo' plants.

On the basis of these data, it was clear that the action of *Trichogramma* wild populations alone against ECB eggs infestation was not effective enough to keep damage to production under acceptable levels.

Figures 1a and 2a display similar trends of both ECB oviposition and *T. brassicae* parasitism rates in 2006 and 2007, respectively. No ECB oviposition was recorded until the second half of July. Then the number of egg masses grew slowly until a sharp increase was recorded in August. Finally, this growing trend reversed in September.

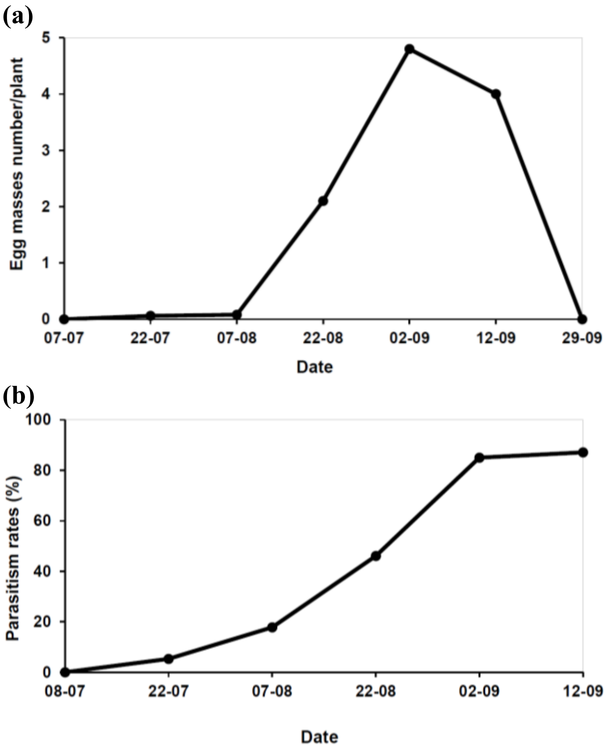

Figure 1. Trend of ECB oviposition (**a**) and parasitism rates (**b**) by *T. brassicae* recorded in 2006.

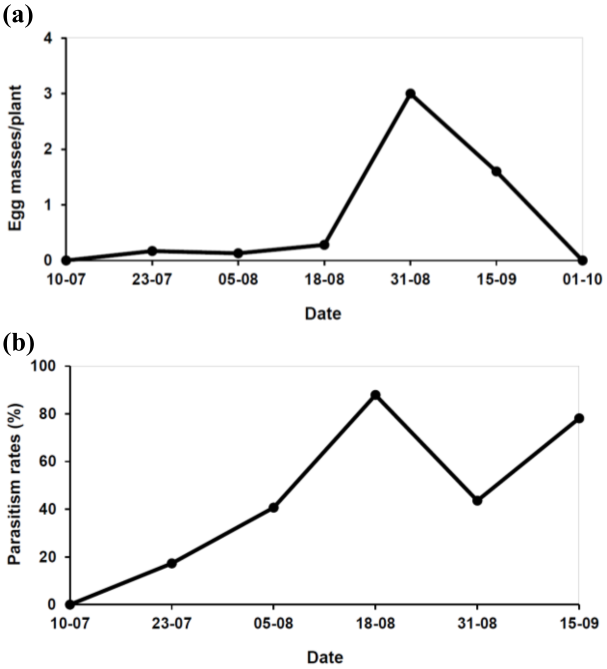

Figure 2. Trend of ECB oviposition (**a**) and parasitism rates (**b**) by *T. brassicae* recorded in 2007.

The growth of egg masses parasitism is shown in figures 1b and 2b. Except for a temporary decrease recorded at the end of August 2007, parasitism rates tended to gradually rise, peaking in September. It has to be noticed that on 20th and 21st August 2007 temperature dropped to values significantly below seasonal average and strong winds were recorded (34 km/h, Osservatorio meteo Voghera).

Experiment 2: use of *Btk* (season 2008)

A different density of ECB egg masses on plants of compared landraces was confirmed by 2008 samples (χ^2 test, $P < 0.01$) when 'Voghera' ($N = 17$) hosted a number of eggs significantly lower than the one recorded on 'Cuneo' ($N = 37$) and 'Asti' ($N = 49$) landraces.

Actually, ECB female moths showed a significant preference ($P < 0.01$) for sweet pepper plants included in control plots, where out of 103 egg masses sampled, 76 were found on plants in untreated plots (χ^2 test, $P < 0.01$).

Egg masses oviposition and parasitism trends were in line with the ones already observed in 2006 and 2007 (figure 3a-b): when the abundance of egg masses tended to rise, levels of parasitism also grew. If *Btk* plots are compared to untreated ones, parasitism rates are not significantly different starting from the half of August. It has to be noticed that a lack of parasitism occurred at the beginning of sweet pepper colonization by ECB in *Btk* plots.

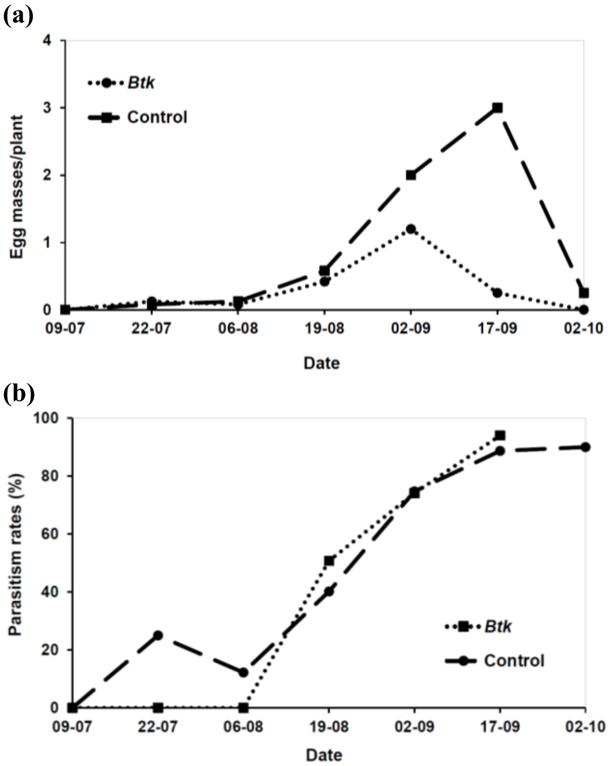

Figure 3. Trend of ECB oviposition (**a**) and parasitism rates (**b**) by *T. brassicae* recorded in 2008.

Table 4 summarizes data on damage to crop production. Undamaged fruit production from 'Cuneo', 'Voghera' (P < 0.05) and 'Asti' (P < 0.01) plots was higher in *Btk* plots compared to control ones (Mann Whitney test) for each one of compared landraces. Similar results came from the pairwise comparison test applied to damaged production (Mann Whitney test; P < 0.05) except for 'Cuneo' landrace.

Foliage biomass ('Asti' 150.9 ± 15.9 g; 'Cuneo' 149 ± 20.1 g; 'Voghera' 134 ± 10.3 g) recorded at the end of the growing season was not significantly different (Mann Whitney pairwise test; P > 0.05).

In control plots a significant correlation occurred between the number of ECB egg masses laid on plants and plant foliage biomass (N = 24; Pearson's correlation r = 0.79; P < 0.01). Such a trend does not appear when the same kind of statistical analysis was applied to data coming from *Btk* plots (N = 24; r = 0.15; P > 0.05).

Experiment 3: comparison trials *Btk* vs deltamethrin (season 2009)

The scarce preference of 'Voghera' plants as oviposition sites (N = 36 egg masses) versus compared landraces ('Asti' N = 59; 'Cuneo' N = 55) was confirmed by data achieved in 2009 (χ^2 test; P < 0.05).

Oviposition trends by ECB are displayed in figure 4a; the maximum increase of ECB egg masses occurred earlier than in previous trials. In figure 4b rapid rising of parasitism trend can be observed. Parasitism rate was on average 74.4% in deltamethrin plots versus 77.9% in *Btk* plots. Those values were not significantly lower in comparison with 86.2% in control plots (χ^2 test, P > 0.05).

No significant difference (Mann Whitney pairwise test; P > 0.05) occurred among landraces in relation to fresh foliage biomass ('Asti' 254.5 ± 22.5 g; 'Cuneo' 221,8 ± 21.5 g; 'Voghera' 280.4 ± 26.7 g). In addition, no significant relation was recorded between the amount of fresh biomass of plants and the number of egg masses laid on them (N = 35; r = −0.12; P > 0.05).

Data on crop loss due to ECB are summarized in table 5. Net productions in control area was not different from the one recorded in treatment plots (Mann Whitney test, P > 0.05) while damaged production was higher in control plots if compared to both *Btk* and deltamethrin plots for each of tested landraces (Mann Whitney test, P < 0.05). No significant difference in terms of damaged production occurred when *Btk* plots are compared to deltamethrin ones.

Table 4. Crop production ± S.E. (damaged + undamaged fruits - year 2008).

Landrace	Trial	Production/plant (g)	Production loss (%)
'Asti'	Control	1034.4 ± 257.3	62.9
'Asti'	*Btk*	1489 ± 143.5	10.3
'Cuneo'	Control	1052.8 ± 112.3	44.6
'Cuneo'	*Btk*	1056.2 ± 112.7	15.6
'Voghera'	Control	1189.2 ± 68	18.9
'Voghera'	*Btk*	1214.1 ± 157.4	1.6

Table 5. Crop production ± S.E. (damaged + undamaged fruits - year 2009).

Landrace	Trial	Production/plant (g)	Production loss (%)
'Asti'	Control	1240.7 ± 273.3	26.5
'Asti'	*Btk*	1296 ± 198.5	5.2
'Asti'	Deltamethrin	1134.4 ± 98.1	3.4
'Cuneo'	Control	1237.7 ± 259.3	34.1
'Cuneo'	*Btk*	1438.2 ± 240.6	4.8
'Cuneo'	Deltamethrin	1162.5 ± 120.5	6.1
'Voghera'	Control	1443 ± 138.8	26
'Voghera'	*Btk*	1742.1 ± 128.2	2
'Voghera'	Deltamethrin	1366.1 ± 182.8	7.2

Table 6. Crop production ± S.E. (damaged + undamaged fruits - year 2010).

	N (sample plants)	Production/plant (g)	Damaged production (%)
Control	15	854.1 ± 61.7	15.1
Btk	15	904.5 ± 91	2.8
Deltamethrin	16	840 ± 88.2	4.8

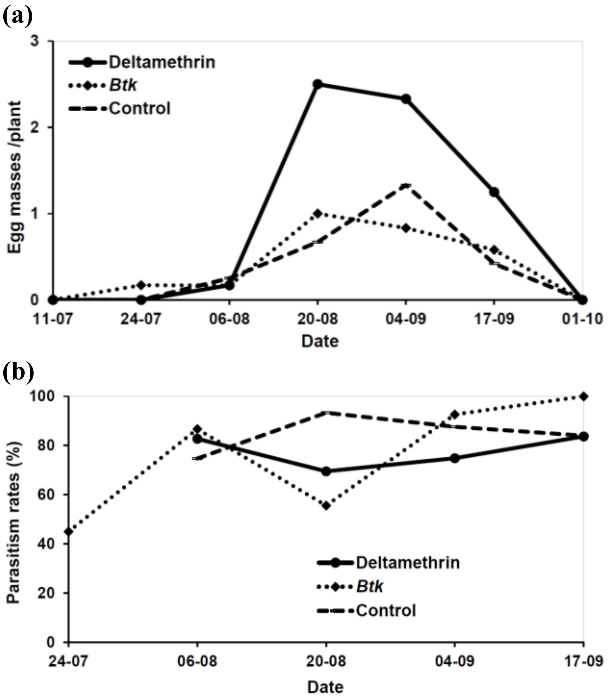

Figure 4. Trend of ECB oviposition (**a**) and parasitism rates (**b**) by *T. brassicae* recorded in 2009.

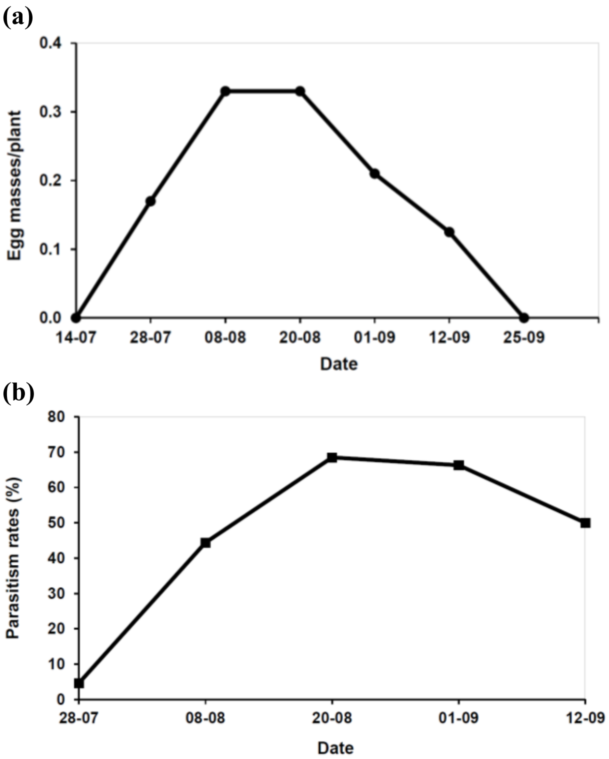

Figure 5. Trend of ECB oviposition (**a**) and parasitism rates (**b**) by *T. brassicae* recorded in 2010.

The results obtained by trials carried out in the farm located next to Voghera were consistent with the ones resulting from experimental plots. Average parasitism rate recorded in control area (67.5%) was significantly higher (χ^2 test, $P < 0.05$) than the one observed in the field sector where deltamethrin was sprayed (48.9%). On the contrary, data on average parasitism rate from *Btk* spray area (64.2%) was not significantly different from the one from control sample (χ^2 test, $P > 0.05$). The effect of deltamethrin on *T. brassicae* can be related to the acute toxicity induced by this active ingredient (Thubru *et al.*, 2018) and the alteration of behaviour due to sublethal doses (Delpuech *et al.*, 1999; Delpuech and Delahaye, 2013). Ksentini *et al.* (2010) demonstrated that, even six days after deltamethrin treatments, the lethal residues on pomegranate leaves affected three different *Trichogramma* species.

It has to be emphasized that two *Trichogramma* species emerged from ECB eggs, *T. brassicae* and *Trichogramma agrotidis* Voegele et Pintureau. The record of *T. agrotidis* from *O. nubilalis* is the first one in Italy; this species in the past was observed in Switzerland, France, Bulgaria and Russia (Pintureau, 2008).

Experiment 4: final testing of *Btk* (season 2010)

ECB oviposition trend is displayed in figure 5a. The shape of the graph is not consistent with the ones achieved in previous years of trials. Reasonably due to the longer distance separating experimental plots from the closest maize field (> 500 m), egg masses abundance was quite poor. *Trichogramma* species was regularly recorded, but - probably in response to low ECB egg masses density - the parasitism rates do not reach as high levels as the ones recorded in previous years (figure 5b).

As a consequence of poor ECB egg masses density (figure 5a) low levels of crop losses were observed. Good results came from the application of *Btk* (table 6). Damaged production from *Btk* samples was significantly lower in comparison with the one from untreated control (Mann Whitney test, $P < 0.05$).

Egg masses oviposition and parasitism by *T .brassicae* in relation to the distance from maize fields

Table 7 summarizes the results of ECB egg masses parasitism on sweet pepper and neighbouring maize fields. Values achieved from maize and pepper have been pairwise compared.

The general trend showed a higher efficacy of the parasitoid in maize than in sweet pepper plots, in spite of the fact that the area of foliage to explore is much greater; such a difference complies with results by Kuhar *et al.* (2004) who compared the activity of *Trichogramma ostriniae* Pang et Chen on maize and solanaceous crops. Maini *et al.* (1991) found differences in *T. brassicae* parasitism on sentinel ECB egg masses set up in pepper plants

Table 7. Parasitism rates (%) of ECB egg masses from both sweet pepper plots and maize recorded in the first half of August (I), in the second half of August (II) and in the first half of September (III). Maize fresh silk can be observed in July, before period I. Data concerning maize/sweet pepper coming from the same year and the same period (I, II, or III column) are compared, (*) indicates significant differences (P < 0.05 - contingency tables).

Year	Crop	I	II	III
2007	Maize	55.3*	69.5*	56.7*
2007	Sweet pepper	40*	35.5*	78.3*
2008	Maize	15.3	61*	82*
2008	Sweet pepper	16.2	43.9*	74.6*
2009	Maize	71.4*	69.1*	80.3
2009	Sweet pepper	58*	59.4*	75

compared to maize plants, speculating a correlation with the different foliage area of maize vs pepper.

Parasitism rates from wild species of *Trichogramma* populations on both crops (maize and sweet pepper) were higher than the ones recorded by Burgio and Maini (1995) in the central Po floodplain, while data concerning maize were consistent with the ones achieved by past researches carried out in the same area where sweet pepper plots were organized (Camerini *et al.*, 2018).

With regard to parasitism rates by *T. brassicae*, after the colonization of sweet pepper by the egg parasitoids, their efficiency in searching EBC tended to rise over time (r = 0.79, P < 0.01). Such a pattern was common to the years 2006-2009, when ECB egg masses were significantly more numerous due to the shorter distance of maize fields from sweet pepper plots (figure 6).

The evolution of eggs number/egg mass shows an opposite trend (r = −0.57; P < 0.01) (figure 7); egg masses size laid at the end of ECB life cycle tended to be smaller (figure 7).

The results confirm the role of maize cultivations as primary reservoir of ECB infestations. The distance separating maize and sweet pepper fields and the extent of land devoted to maize production within an agroecosystem could be an important factor affecting potential damage to sweet pepper cultivations. The importance of distance was proved by the significant difference between ECB egg masses density recorded in sweet pepper plots in 2006-2009 trials (distance < 150m) and the one observed in 2010, when sweet pepper plots were separated 500 m from maize fields.

In the study area sweet pepper transplantations occur in June and for quite a long period ECB are poorly attracted by sweet pepper plants, mainly due to the incomplete development of foliage biomass and limited availability of ripening fruits. This is in accordance with the observation that ECB larvae are not attracted by small sized sweet pepper fruits (Hitchner and Ghidiu, 2006).

In the Po river valley oviposition by EBC females on sweet pepper plants starts in July, but usually egg masses density was found to be low until middle August, when it tended to increase rapidly. Such a tendency corresponds to the late ripening stage of maize, whose stalk, leaves and ears start to show clear symptoms of desiccation. Those changes stimulate ECB adult females to disperse around in search for other crops to colonize. A similar timing of damage appearance in sweet pepper cultivations was recorded in Austria by Kahrer (2005).

'Voghera' sweet pepper proved to be less exposed to damage in comparison to 'Asti' and 'Cuneo' landraces. Sweet pepper susceptibility to ECB attacks is affected by several factors, including fruit size and shape, and spiciness (Frantz *et al*., 2004; Kahrer, 2005; Hitchner and Ghidiu, 2006; Larue and Welty, 2010). Those factors cannot be taken into account to explain the different susceptibility of the landraces compared by this study. 'Voghera', 'Asti' and 'Cuneo' landraces do not significantly differ in terms of fruit size, shape and pungency, indeed. 'Voghera' fruiting is slightly earlier than the one of compared landraces, but this property can only partially explain the lower occurrence of ECB egg masses laid on 'Voghera' pepper plants. The hypothesis tested here is that the extent of foliage biomass of every plant - regardless of the landrace it belongs to - could affect ECB females laying. Actually, a positive relation between foliage fresh biomass of the plant and abundance of egg masses was recorded in 2008, but it was not confirmed in 2009. In addition, this factor cannot justify the higher levels of damage suffered by 'Cuneo' and 'Asti' landraces, because no significant difference in terms of foliage biomass was demonstrated when samples of plants of different landraces were compared.

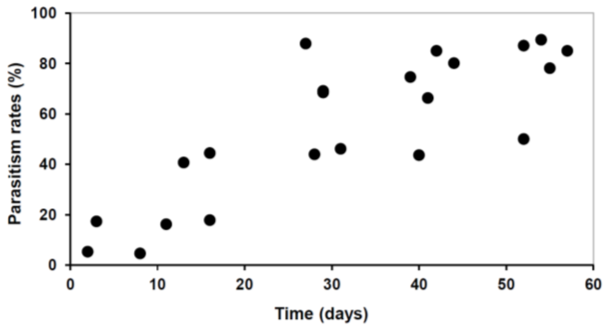

Figure 6. Parasitism rates trend. 20th July has been fixed as the first day of ECB oviposition season (years 2006-2009).

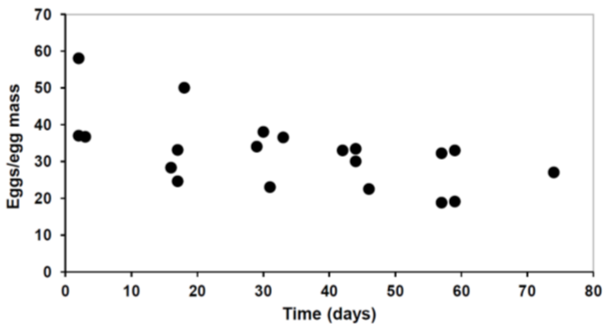

Figure 7. Number of ECB eggs/egg mass. 20th July has been fixed as the first day of ECB oviposition season (years 2006-2009).

A possible influence of semiochemicals emitted by plants or rotten fruits can be hypothesized; data achieved in 2008, when much more egg masses were recorded in control plots in comparison with treated ones could support such a hypothesis, which needs to be further explored, given that data coming from 2009 and 2010 did not confirm such a trend.

Conclusions

Even if *T. brassicae* and *T. agrotidis* by themselves cannot limit ECB population under the economic damage threshold, it has to be emphasized that, after an initial delay, their egg parasitoid populations can rise in response to ECB eggs increase, so giving an important support to a biocontrol strategy based on *Btk* application.

T. agrotidis can also attack ECB egg masses: this is the first record of this species in Italy.

Findings of these field trials can suggest a protocol to be applied when both sweet pepper and maize are grown in the same agroecosystem and are close to each other, since this proximity poses a significant threat to sweet pepper production. Damage threshold on a cash crop like pepper is very low: one single ECB larva can damage several sweet pepper fruits during its life cycle. As long as early fruits have not reached a consistent size, ECB is not a real threat, but - as this case of study demonstrates - as soon as early fruits are going to ripen, ECB females start to lay egg masses. Sweet pepper cultivations tend to become particularly sensitive to ECB attack when maize in agroecosystem ripens and most of foliage biomass tends to dry. In this growing stage maize ceases to be attractive for ECB females searching for oviposition sites, while the availability of sweet pepper cultivations next to maize fields offers favourable chances to support ECB life cycle. In such a condition, the only natural control coming from *Trichogramma* species wild populations cannot by itself limit ECB under damage threshold, therefore *Btk* sprays are as well needed to preserve sweet pepper production.

The setup of traps baited with sex attractant and PAA can help to synchronize the beginning of *Btk* spray with the start of sweet pepper cultivation colonization by ECB females, since mated females trapping can work as an alarm signal for *Btk* first application. In any case, given the poor persistency of *Btk* toxins, the efficacy of sprays was strictly related to treatment frequency: good results came from the application of *Btk* every six days.

The effectiveness of the protocol can be further verified by the weekly dissection of a random sample of 20 ripe fruits. When at least one fruit is found invaded by ECB larvae this can be an indication that the economic damage threshold has probably been over.

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Authors' addresses: Giuseppe CAMERINI (corresponding author, e-mail: giuseppe.camerini@gmail.com, giuseppe.camerini@taramellifoscolo.it), Istituto di Istruzione Superiore "Taramelli-Foscolo", via Mascheroni 53, 27100 Pavia, Italy; Monica MASANTA, Istituto di Istruzione Superiore "A. Maserati", via Mussini 22, 27058 Voghera, Italy; Stefano MAINI, Department of Agricultural and Food Sciences, *Alma Mater Studiorum* Università di Bologna, viale G. Fanin 42, 40127 Bologna, Italy.

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