Crop Protection 142 (2021) 105529

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Sustainability of strategies for *Ostrinia nubilalis* management in Northern Italy: Potential impact on beneficial arthropods and aflatoxin contamination in years with different meteorological conditions

Serena Magagnoli [a,*], Alberto Lanzoni [a], Antonio Masetti [a], Laura Depalo [a], Marco Albertini [b], Roberto Ferrari [c], Giorgio Spadola [d], Francesca Degola [d], Francesco M. Restivo [d], Giovanni Burgio [a]

[a] *Alma Mater Studiorum-Università di Bologna (UNIBO), Dipartimento di Scienze e Tecnologie Agro-Alimentari (DISTAL), Viale G. Fanin 42, 40127, Bologna, Italy*
[b] *Agrites S.r.l., Via Marconi 4/2, 40057, Granarolo dell'Emilia (BO), Italy*
[c] *C.A.A. "Giorgio Nicoli" S.r.l., Via Sant'Agata 835, 40014, Crevalcore (BO), Italy*
[d] *Università degli Studi di Parma (UniPR), Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale (SCVSA), Parco Area delle Scienze 11/a, 43124, Parma, Italy*

ARTICLE INFO

Keywords:
Ostrinia nubilalis
Integrated pest management (IPM)
Organic farming
Aflatoxins (AFB_1)
Biological control
Biopesticide

ABSTRACT

The European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is a key pest of maize (*Zea mays* L.). In Northern Italy, larvae of the 2nd generation may damage cobs and kernels and the feeding activity can promote the proliferation of aflatoxigenic fungi, such as *Aspergillus flavus*, the major producer of aflatoxin B_1 (AFB_1).

During 2017–2018, the efficacy of two strategies to control ECB in maize was assessed. Biological control strategy (*Trichogramma brassicae* Bezdenko and *Bacillus thuringiensis* Berliner), conventional chemical strategy (chlorantraniliprole) and an untreated control were compared to assess: i) the effect of the strategies on ECB infestation; ii) the association of ECB and AFB_1 kernels contamination; iii) the impact of the strategies on beneficial arthropods.

The conventional chemical strategy demonstrated the best control of ECB infestation, followed by biological control strategy and the untreated control. No significant differences in maize yield were found among strategies. The role of ECB on AFB_1 concentration was demonstrated only in 2017, when higher level of infestations occurred simultaneously with an extended period of drought and high temperatures, sanctioning the important role of meteorological conditions on AFB_1 contamination.

The activity density of ground beetles, rove beetles and spiders and the mean number per leaf of the most abundant beneficial insects dwelling on plants (coccinellids, predatory thrips and lacewings) did not show significant changes between pre-and post-treatment with chlorantraniliprole, highlighting the selectivity of this pesticide in the short time.

This study provides some contribution for the reduction of non-renewable input in Italian maize fields, demonstrating that biological control strategy, although less effective than conventional chemical control, can be a feasible approach to control ECB second larval generation, without any increment of AFB_1 level in grains and yield loss.

* Corresponding author. *Alma Mater Studiorum*-Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari (DISTAL), Viale G. Fanin 42, 40127, Bologna, Italy.

E-mail addresses: serena.magagnoli4@unibo.it (S. Magagnoli), alberto.lanzoni2@unibo.it (A. Lanzoni), antonio.masetti@unibo.it (A. Masetti), laura.depalo@unibo.it (L. Depalo), albertini.intesia@agrites.it (M. Albertini), rferrari@caa.it (R. Ferrari), giorgio.spadola1@studenti.unipr.it (G. Spadola), francesca.degola@unipr.it (F. Degola), francescomaria.restivo@unipr.it (F.M. Restivo), giovanni.burgio@unibo.it (G. Burgio).

https://doi.org/10.1016/j.cropro.2020.105529
Received 28 September 2020; Received in revised form 22 December 2020; Accepted 23 December 2020
Available online 30 December 2020

Site	Year	Coordinates
1	2017	44°28'37.1"N 11°28'55.2"E
2	2017	44°32'03.4"N 11°07'49.4"E
3	2018	44°24'38.79"N 11°32'32.01"E
4	2018	44°32'4.10"N 11° 7'47.09"E

Fig. 1. Geographic location and coordinates of the four sampling sites monitored during the two-years study.

1. Introduction

Maize (*Zea mays* L.) was domesticated approximately 9000 years ago in Iowlands of the Central Balsas (River Valley, Mexico) becoming one of the most cultivated crops all over the world (Leff et al., 2004; Warburton et al., 2011). In Italy, maize is grown in 660,000 ha with a production of more than 6 million tons per year (FAOSTAT, 2016). Northern Italy is the most important maize production area in Italy, with an intensive crop output in the Po Valley region (Meissle et al., 2010). During the last century, maize yields increased markedly by means of extensive monoculture, high-productive hybrids, and intensive agricultural practices based on the use of non-renewable inputs such as pesticides, herbicides and fertilizers. All these factors contributed to detrimental impacts on the environment, including landscape simplification and loss of biodiversity (Tilman, 1999). In the recent years much effort has been made in decreasing the environmental impact of agricultural practices; nevertheless, matching productivity and sustainability does not have any easy solutions (Tilman, 1998).

The European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) is one of the key pests of maize. In Northern Italy this pest usually completes two generations per year: during spring, the first larval generation is responsible for negligible damages on leaves, whereas in summer larvae of the second generation feed on cobs and kernels (Windham et al., 1999). A third partial generation can sometimes occur when meteorological conditions are favourable (Alma et al., 2005; Camerini et al., 2015). In many European countries, ECB conventional control relies on foliar spray of broad-spectrum synthetic insecticides, with well-known side effects including negative impacts on non-target organisms and the risk of resistance development (Vasileiadis et al., 2017). Sustainable alternatives are currently available in order to reduce the use of synthetic insecticides in maize cultivations, including the application of the microbial agent *Bacillus thuringiensis* var. *kurstaki* Berliner (Bravo et al., 2011; Sanchis, 2011) and the egg parasitoids belonging to genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae), that have been used since the mid 80s (Bigler, 1986; Burgio and Maini, 1995; Hassan et al., 1978; Razinger et al., 2016; Voegele, 1975).

Besides insect damages, maize is prone to infection by various pathogens, including *Aspergillus* and *Fusarium* species. Some of them are well known producers of different mycotoxins, whose impact on both human and animal health may eventually lead to significative economic losses (Fernández-Ibañez et al., 2009; Camardo Leggieri et al., 2015). The most common mycotoxins in pre-harvested maize are aflatoxins, mainly produced by *Aspergillus flavus* Link and *A. parasiticus* Speare, together with fumonisins produced by *Fusarium verticillioides* (Sacc.) Nirenberg and *Fusarium proliferatum* (Matsush) Niremberg. Among aflatoxins, B_1 (AFB_1) is considered as the most toxic and carcinogen natural compound, being classified by the International Agency for Research on Cancer (IAARC), as class-1 human carcinogen (Klich, 2007; Windham et al., 1999). Its level in food and feedstuffs is strictly monitored and regulated (Cheli et al., 2014; Esmaeilishirazifard and Keshavarz, 2014). In particular, the European Commission have established a regulatory limit of AFB_1 equal to 0.02 ppm in maize intended for animal feedstuffs, as reported in the Commission Regulation N° 1881/2006 (European Commission, 2006). The prevalence of one mycotoxigenic species over the others is strictly related to the environmental and climatic conditions, mostly to temperature and humidity. High temperatures and late-season precipitation induce drought stress in maize plants, promoting the *A. flavus* spread, whereas warm conditions and high humidity are critical for the diffusion of *Fusarium* spp. (Battilani et al., 2016). In the recent years, a shift towards aridity occurred in the Po Valley region, creating more favourable conditions for *Aspergillus* spp. diffusion and, therefore, for aflatoxins production (Battilani et al., 2016).

Although soil is not the primary habitat for *Aspergillus* spp., spores and other reproductive structures (e.g. sclerotia) might overwinter at the ground level in plant debris. Then, in presence of favourable environmental conditions, they can germinate originating mycelium and producing, at the end of the fungal life cycle, other spores, which are spread by wind and insects in the surrounding environment (Abbas et al., 2009; Coppock et al., 2018). A number of both abiotic and biotic stresses, such as drought and insect damages, have been associated with contamination of aflatoxins (Chen et al., 2004; Cotty and Jaime-Garcia, 2007; Payne and Brown, 1998; Windham et al., 1999). However, despite association of insect damages and aflatoxins contamination have been reported as an important factor for the fungal colonization (Blandino et al., 2015; Widstrom, 1979; Windham et al., 1999), the correlation between ECB and AFB_1 concentration in grains is still debated, probably due to the strong influence of environmental conditions on the aflatoxin metabolism in the fungus (Abbas et al., 2009; Guo et al., 2008; Klich, 2007; Sanchis and Magan, 2004).

The ubiquitous occurrence of toxigenic *Aspergillus* spp. in the fields (Abbas et al., 2009) and the demonstrated toxicity of aflatoxins, strongly demands for the development of effective strategies for the reduction of aflatoxins contamination in maize. Many approaches have been suggested to reduce pre-harvest contamination including the selection of

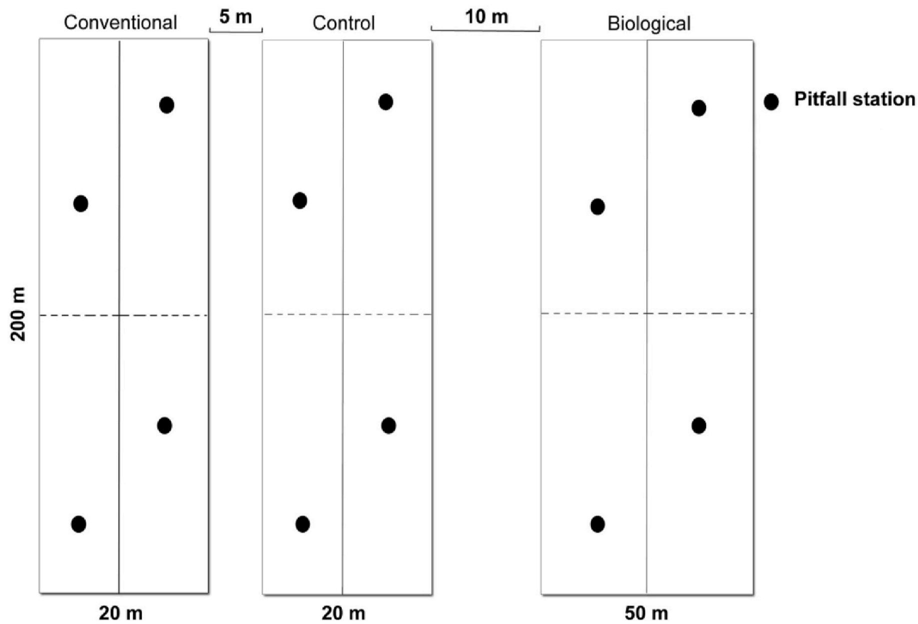

Fig. 2. Experimental field scheme, which was established in each site, with indications of the three strategies and relative sub-plots division. Black dots: pitfall stations used to monitor ground-dwelling arthropods in field.

maize resistant varieties, the mitigation of plant stresses and the use of chemical and biological agents (Umesha et al., 2017).

The general aim of this two-year field experiments was to compare a conventional chemical ECB control strategy, based on the use of a synthetic insecticide, with a biological control strategy, in order to provide the best practice guideline for reducing chemical inputs of maize agro-ecosystems in Northern Italy. The insecticide chlorantraniliprole was selected as chemical standard, because it is currently one of the most used products in integrated pest management in Northern Italy; the combined use of the parasitoid *Trichogramma brassicae* Bezdenko and the microbial agent *B. thuringiensis* var. *kurstaki* was chosen for the biological control strategy. Specific aims of this work included: i) a comparative analysis of ECB infestation, maize yield and AFB_1 production among the different plant protection strategies; ii) an assessment of the impact of control strategies on beneficial fauna, including ground dwelling and canopy arthropods.

2. Material and methods

2.1. Study sites and experimental design

The surveys were carried out during two consecutive years (2017-18) in four fields (two per year) located in Bologna province, Northern Italy (Fig. 1). In each experimental field (2.2 ha; 110 × 200 m) three plots were delimited (Fig. 2). Three different strategy for ECB control were tested:

i.) Conventional chemical, based on a single application of Coragen® (active ingredient: chlorantraniliprole 200 g/L; Cheminova Agro Italia Srl) at field dose of 125 mL/ha in 2 hL/ha of water;
ii.) Biological control, combining Turex® (Bt) (composition: *B. thuringiensis kurstaki*-HD1 and *B. thuringiensis aizawai*-H7 25.000 U.I./mg; SCAM Spa) at field dose of 1 kg/ha in 2 hL/ha of water and Triko250® (Bioplanet Sca)/Trichosafe® (De Sangosse Italia Srl). Both products are composed by *Trichogramma brassicae* and approximately 400.000 individuals/ha were released;
iii) Untreated (control) where no control measures were undertaken.

Conventional chemical and biological control strategies were planned on the basis of the results of pheromone traps. Once the peak of ECB adult flight was reached, treatments were scheduled as soon as possible, taking into account the machineries availability and the weather forecast. Four sub-plots per each plot were established in each field, in order to optimize the data collection. Coragen® and Turex® were sprayed on plants by an air-assisted sprayer (Model Gaspardo Uragano 3000). *T. brassicae* were released in field by drone (DJI Matrice 100) in the form of parasitized eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) inside biodegradable cellulose capsules. The drone was programmed in order to follow a predefined path and to automatically release the biological agents in field. Two releases of *Trichogramma* wasps were carried out in 2017, whereas due to a delay in ECB adult peak emergence, caused by abundant precipitation and mild temperature, only one drone flight was conducted in 2018. Suverkropp et al. (2009) reported a very short flight distance of *T. brassicae* from the release point, therefore a buffer zone of 10 m separated the biological control plots from other treatments. The timing of each treatment is reported in supplementary materials.

2.2. Maize variety and experimental planning

Year 2017 (Sites 1 and 2): Maize hybrid Pico AMERICAN® genetics (FAO 400, 7 seeds/m2) was sown on 11th-13th April in Site 1 and on 14th April in Site 2. The pre-emergence herbicides Gallup biograde® 360 (active ingredient: glyphosate 360 g/L; at field dose of 4 L/ha; Barclay Chemicals (R&D) Ltd) and Adengo® (active ingredient: thiencarbazone-metyl 20 g/L, isoxaflutole 50 g/L, cyprosulfamide 33 g/L; at field dose of 2 L/ha; Bayer CropScience) were spread on soil immediately after sowing. Nutrifos Hp (300 kg/ha; SCAM S.p.a.) was applied as pre-sowing fertilizer, while the coverage nitrogen fertilization was performed with urea (46% N; 500 kg/ha). Three irrigations were carried out from mid-June to the end of July by a travelling gun irrigation system for a total of 150 mm of water (50 mm each).

Maize was mechanically harvested (Model John Deere S650) on 22nd August (BBCH 99) in Site 1 and on 23rd August in Site 2 (BBCH 99). Grains obtained from each sub-plot (four per strategy) were weighted by using a truck weighing balance (Model DFWKR).

Year 2018 (Sites 3 and 4): Maize hybrid Helium Syngenta® (FAO 400, 7.5 seeds/m2) was sown on 20th-23rd April in Site 3 and 4

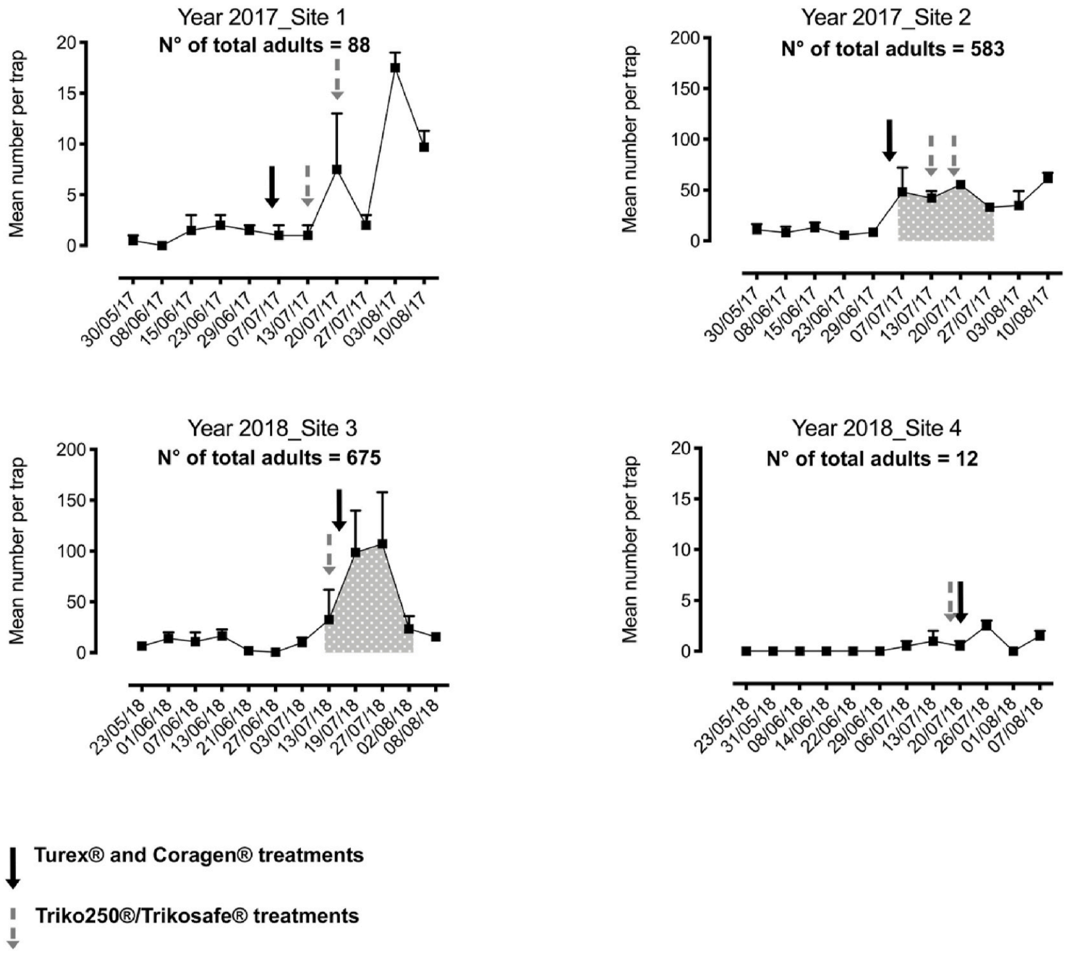

Fig. 3. Trend of adult ECB caught by pheromone traps. The second flight of ECB was identified by monitoring two pheromone traps per field. Treatments were planned on the basis of the results of Sites 2 and 3 due to the high number of adults caught. Black arrows indicate the application of Coragen® and Turex®, while grey dotted arrows correspond to Triko250®/Trichosafe® release. Vertical lines indicate the standard error of the means.

respectively. The pre-emergence herbicides Gallup biograde® 360 (active ingredient: glyphosate 360 g/L; at field dose of 5 L/ha; Barclay Chemicals (R&D) Ltd) and Adengo® (active ingredient: thiencarbazone-metyl 20 g/L, isoxaflutole 50 g/L, cyprosulfamide 33 g/L; at field dose of 2 L/ha; Bayer CropScience) were applied on soil immediately after sowing. Nutrigran top (300 kg/ha; SCAM S.p.a.) was applied as pre-sowing fertilizer, while the coverage nitrogen fertilization was carried out with urea as in the previous year. Three irrigations were carried out from mid-June to the end of July by using a travelling gun irrigation system for a total of 120 mm of water (40 mm each).

Maize was mechanically harvested (Model John Deere S650) on 30th August (BBCH 99) in Site 3 and on 31st August (BBCH 99) in Site 4. Grains obtained from each sub-plot (four per strategy) were weighted by using a truck weighing balance (Model DFWKR).

Daily data of rainfall (mm) and temperature (°C) were obtained from Dexter3r regional service (https://simc.arpae.it/dext3r/) in order to check a possible influence of meteorological conditions during the sampling period. Meteorological stations were selected as close as possible to the experimental fields.

2.3. Arthropod samplings

Flight dynamics of Ostrinia nubilalis: Flights of ECB adults were monitored by means of two pheromone traps per field (CORETRAP®). Traps were checked weekly from mid-May to the beginning of August in order to identify the second flight and establish the optimal timing for treatments. The distance between traps was >50 m. Both traps were baited with the sex pheromone lures ((E)-11-tetradecenyl acetate, ISAGRO®; (97:3) E:Z -11- 14Ac 0.1). One of the two traps was also equipped with phenylacetaldehyde (PAA), (ISAGRO®) in order to attract both males and females. Sex-pheromone was replaced every two weeks, while PAA was changed every three weeks.

Ground dwelling arthropod samplings: Ground dwelling arthropods were sampled by means of pitfall stations. Each pitfall station consisted of two cups connected by a plastic barrier (15 × 100 cm); each cup (600 mL, ø 10 cm) was buried at ground level and covered with plastic lid in order to prevent flooding. Cups were filled with approximately 200 mL of 40% aqueous solution of propylene glycol. Pitfall stations were activated for a week before the application of insecticide Coragen® and one week after the treatment for a total of 14 days. Overall, 12 pitfall stations per field (4 per strategy, 1 per sub-plot) were activated during each year.

Traps content was sorted in laboratory and arthropods were divided into taxonomic groups and preserved in 70% ethanol. Ground beetles (Carabidae), rove beetles (Staphylinidae) and spiders (Araneae) were selected because of their effectiveness as indicators of environmental changes (Rainio and Niemelä, 2003); activity density (AD) of these taxa was calculated, for a standard period of 7 days, in each strategy and year.

2.4. Visual samplings on canopy insects

Two surveys were carried out: the first one before the application of Coragen® and Bt (pre-treatments) and the second a week after the treatments (post-treatments). Fifteen plants per sub-plot were sampled

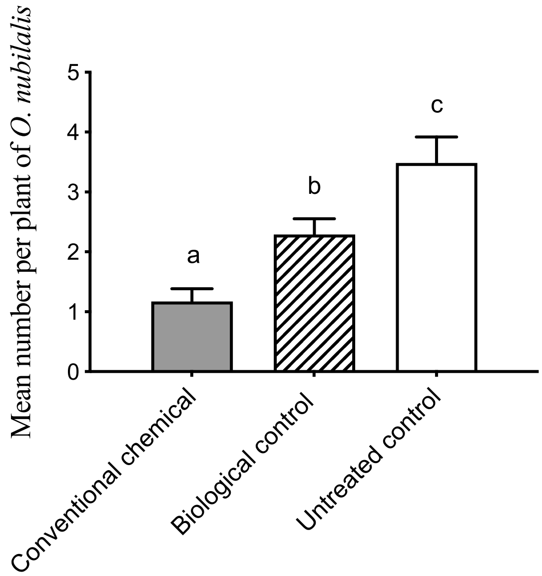

Fig. 4. ECB infestation expressed as mean number of larvae and pupae per plant (+SE) in each strategy (pooled data of two years). Bars bearing different letters are significantly different (ANOVA followed by Ryan-Einot-Gabriel-Welsch multiple comparison procedures; $P < 0.05$).

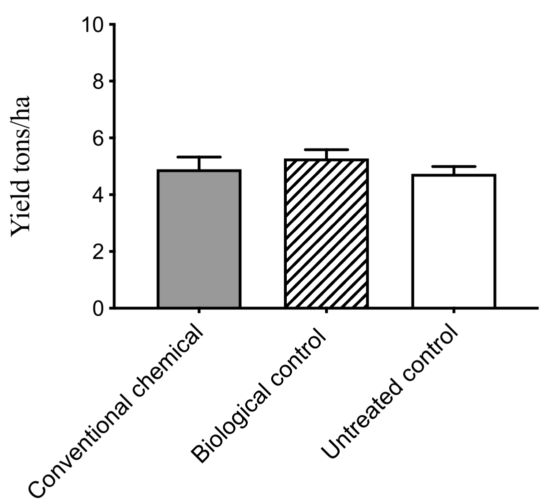

Fig. 5. Maize yield (tons/ha) in each strategy (pooled data of two years). Vertical lines represent + SE of the mean.

by observing three randomly selected leaves for each plant. All the beneficial insects found on leaves were counted during the visual samplings and the mean number per leaf was calculated in each strategy and year.

2.5. ECB infestation

Fifteen randomly selected plants from each sub plot (60 plants per strategy, 180 per field) were sampled to evaluate the infestation by second generation of ECB. Plant stems and cobs were cut, and larvae and pupae of ECB were counted.

Samplings were carried out at the beginning of August in all sites (on 8th August in Site 1 and on 10th August in Site 2 (Year 2017, BBCH 85); on 8th August in Site 3 and on 7th August in Site 4 (Year 2018, BBCH 87).

2.6. Aflatoxin B_1 quantitation in maize kernels

Incremental samples of maize grains (around 100 g each) were randomly and continuously collected during harvest. From the final sample of approximately 5 Kg, 1 Kg was addressed to AFB_1 determination by high pressure liquid chromatographic (HPLC), following the Kobra® Cell method (R-Biopharm Rhône, Ltd). Briefly: 20 g of maize kernels were grounded 3 min in a blender and added with 100 mL 70% MetOH, then filtered through a paper filter, diluted 1:5 in Milli-Q water and filtered with a microfiber filter (1.5 μm, VICAM, Watertown, MA, USA). Ten millilitres of filtrate were passed through immunoaffinity clean-up column (Afla B&G, ORSELL, Modena, Italy), and aflatoxins were recovered by washing the column with 1.5 mL CH_3OH; finally, 0.5 mL of Milli-Q water were added to the flow-through. The chromatographic analyses were performed using a Jasco Model PU-1580 pump, equipped with a Hypersil™ ODS C18 column (250 × 10 mm, Thermo-Fisher Scientific, Waltham, Massachusetts, USA, a Jasco Model AS-1555 autosampler (loop = 0.1 mL), and a Jasco Model FP-1520 fluorescence detector (λ_{ex} = 365 nm and λ_{em} = 440 nm). Run conditions were as follows: mobile phase Water:Acetonitrile:MeOH (72:14:14 v/v/v) with nitric acid and KBr for KOBRA cell; injection volume 400 μL; flow rate 1.2 mL/min.

2.7. Statistical analysis

Mean number of ECB adults per trap and date was calculated in each date and field in order to describe the pest phenology. AD of ground beetles, rove beetles and spiders was calculated as reported by Magagnoli et al., 2018, while the density of canopy insects was expressed as mean number per leaf. An analysis of covariance (ANCOVA) was used to assess potential impact of the strategies on ground dwelling arthropods (ground beetles, rove beetles and spiders) and canopy insects (ladybird beetles, predatory thrips and lacewings). In ANCOVA analysis, the ground-dwelling arthropod AD and the mean number of beneficial per leaf in the pre-treatment were used as covariate, respectively.

ANOVA with blocks was carried out considering the four sites as blocks, in order to test the effect of control strategies (conventional chemical, biological control, untreated control) on ECB infestation (mean number of insects per plant/sub plot) and maize yield (tons/ha). When ANOVA revealed significant differences among treatments, Ryan-Einot-Gabriel-Welsch multiple comparison procedures ($P < 0.05$) was used as post-hoc test. The confidence intervals (95%) method was used to compare AFB_1 concentration (ppm) of each strategy with the corresponding safe limit. Due to the extreme variability in AFB_1 amount between the two growing seasons, aflatoxins charts were drawn separately for each year.

Finally, the role of ECB (mean number of larvae/pupae per plant) in promoting AFB_1 production was tested by means of Pearson correlations. Data of each plot were log transformed and analysed separately for each year. All the statistical analyses were performed with IBM SPSS Statistics (ver. 23).

3. Results

ECB adults caught by traps during both years were in total 1358, of which 1351 were males and 7 females. The second flight of ECB started the first week of July in 2017 and one week later in 2018. Conventional and biological control treatments were planned on the basis of the results of Sites 2 and 3, given that pheromone traps on the other sites caught only a few adults (Fig. 3).

Conventional chemical strategy based on chlorantraniliprole resulted the best one to reduce ECB larval infestation, followed by biological control strategy and untreated control (ANOVA: $F_{2,6} = 10.65$, $P < 0.05$; Fig. 4). No significant differences in ECB infestation were detected among sites (ANOVA: $F_{3,6} = 3.61$, $P > 0.05$). The same statistical analysis carried out on yield showed lack of differences among strategies

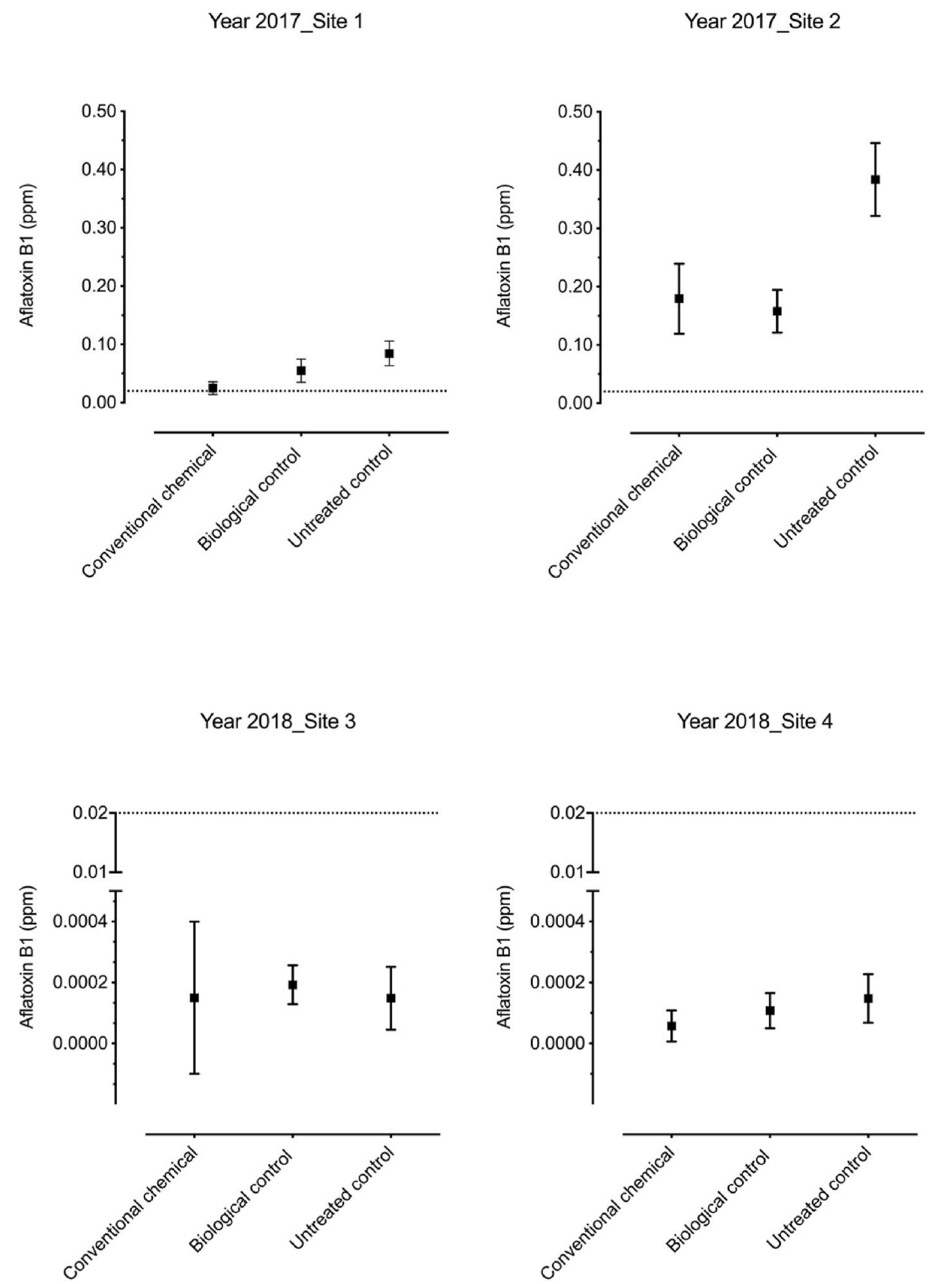

Fig. 6. Mean concentration of aflatoxin B_1 (ppm) as dosed in maize grains from different sites, years and strategies. Vertical lines represent the 95% confidence intervals. The regulatory limit of AFB_1 allowed (0.02 ppm) is indicated by the dotted line.

(ANOVA: $F_{2,6} = 0.57$, $P > 0.05$; Fig. 5).

Confidence interval analysis showed that high levels of AFB_1, above the safe limit allowed by law (0.02 ppm), were detected for year 2017 in all strategies and fields. In particular in 2017 season, the untreated control showed a significant increase in AFB_1 concentration compared with the other strategies in Site 2, instead, in Site 1 the confidence interval of control overlaps with that of biological control strategy but not with that of conventional (Fig. 6). In 2018 AFB_1 were distinctly below the limit (Fig. 6) and the 95% confidence intervals of the strategies overlap each other's in all sites.

A significant positive correlation between the mean number of ECB (larvae and pupae) per plant and the concentration of AFB_1 was found only in Site 2 during 2017 ($R = 0.64$, $P < 0.05$; Fig. 7), while ECB infestation did not impact on mycotoxin concentration in Site 1 in 2017 and in both sites in 2018. The period from 25th May to August 11, 2017 was characterized by extreme hot and dry weather in comparison with historical trends, while in 2018 precipitations and temperature were in line with the seasonal averages. In particular, in 2017 the mean daily seasonal precipitation was 0.6 mm with a mean daily maximum temperature of 32.0 °C, while in 2018 the mean daily seasonal precipitation was 1.8 mm with a mean daily maximum temperature of 29.7 °C (Fig. 8).

The AD of all ground dwelling arthropods (ground beetles, rove beetles and spiders) and the mean number per leaf of canopy beneficial insects did not show any difference among strategies between pre-and post-treatment (ANCOVA, df = 2,9; $P > 0.05$) (Fig. 9).

4. Discussion

Pheromone traps were effective in monitoring adults of ECB and were useful to establish the timing of treatments. However, despite the use of PAA is reported to be effective in attracting females (Maini and

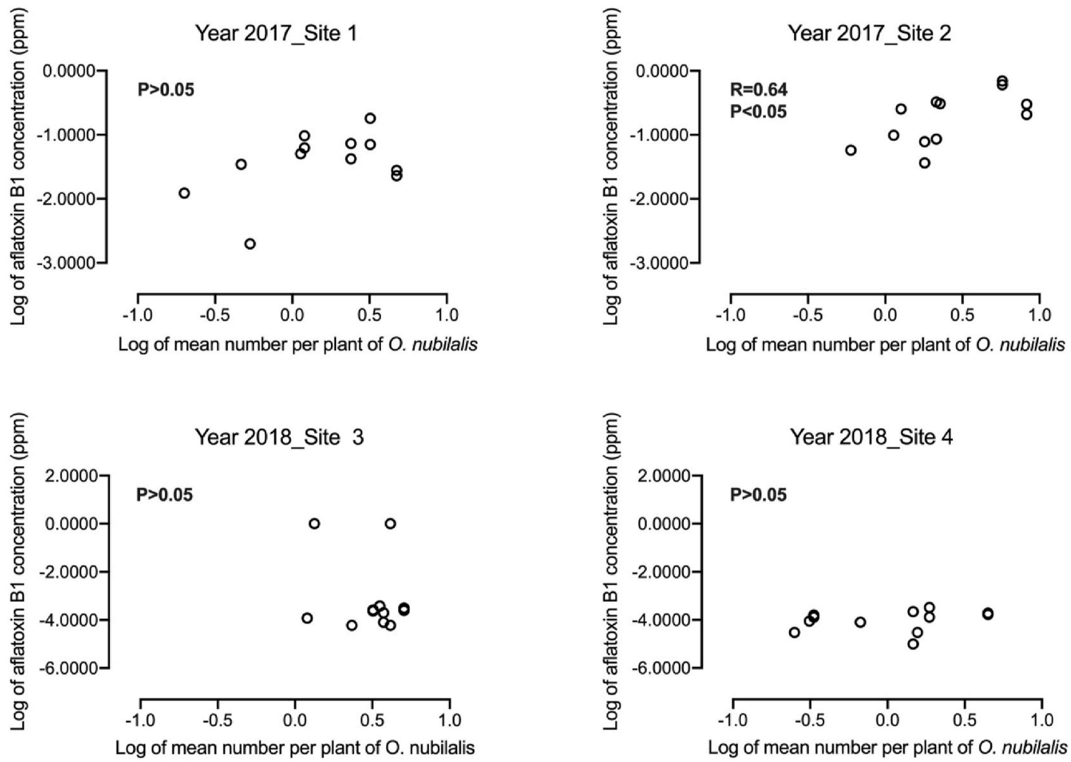

Fig. 7. Pearson correlation between the concentration of aflatoxin B_1 (ppm) in maize grains and the mean number of ECB per plant (larvae and pupae). All data were log10 transformed before evaluation.

Burgio., 1999), we found only seven individuals on a total of 1358 ECB adults. This result is in agreement with that of Tóth et al. (2016), confirming the low effectiveness of low dose PAA on ECB and discouraging its practical use in field.

Conventional approach, based on chlorantraniliprole application, was the best strategy for reducing the ECB second generation larvae. The biological control strategy showed to be more effective in reducing ECB larval infestation than untreated control, though resulting less effective in comparison with chemical strategy. However, despite these results, no significant changes in yields were found among all the strategies. A reduction of pesticide use without any yield loss was also achieved by government programs carried out in Sweden, Canada, and Indonesia, where a decrease of chemicals by 50%–65% was achieved without any yield decrement (Pimentel, 2006).

No strategy was able to constrain the AFB_1 concentration below the allowed threshold during a year characterized by very favourable season for aflatoxin production such as 2017, when temperatures and water deficiency resulted particularly severe. On the other hand, when conditions were less suitable for aflatoxins spread, as in 2018, their level was found significantly lower than the safe limit in all strategies, including the untreated control.

In our study, the abundance of canopy beneficial insects and the AD of ground dwelling arthropods did not differ among strategies in the short time, using an evaluation based on a pre- and post-treatment sampling. However, the lack of harmful activity on predators showed by chlorantraniliprole could be partly due to by the timing of post-treatment samplings, that were carried out a week after pesticide application. While the efficacy on chlorantraniliprole on ECB larvae is already demonstrated and corroborated by this study, its selectivity for beneficial insects remains quite debated. Some authors reported low toxicity of chlorantraniliprole on parasitoids (Brugger et al., 2010; Preetha et al., 2009) and bees (Dinter et al., 2010), but there are also evidences of detrimental effects on *Adalia bipunctata* L. (Coleoptera: Coccinellidae) (Depalo et al., 2017), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) (Gontijo et al., 2014) and *Bombus terrestris* L. (Hymenoptera: Apidae) workers (Smagghe et al., 2013).

Directives and Regulations included in the "Pesticide Package" and adopted by the EU Parliament promote plant protection methods with the minimum impact on agroecosystems. This means promoting and enhancing natural pest control mechanisms through, for example, biological control strategies (Meissle et al., 2010; Vasileiadis et al., 2011). For example, the integration of *T. brassicae* and *B. thuringiensis* seems to be a very promising strategy for the biological control of the second generation of ECB in maize fields. Even if the efficacy of biological and microbial control is known to be influenced by pest density, climatic variables and local receiving environment, the combination of *B. thuringiensis* and *Trichogramma* wasps has emerged as a safe and effective strategy for pest control in many agricultural environments (Hwang et al., 2010; Oatman et al., 1983).

The high variability of climatic trends between the two years of investigation seems the main driver of different AFB_1 levels in maize grains in our field trials. Drought stress associated with high temperature is reported as one of the most important factors that may trigger aflatoxin contamination (Camardo Leggieri et al., 2015; Medina et al., 2014; Payne and Widstrom, 1992). Therefore, the severe drought occurred in 2017 together with the high temperature likely contributed to AFB_1 increase, with strong repercussion on maize quality. Such stressing conditions may be also responsible of "silk cut" symptoms with pericarp rupture and subsequent fungal colonization (Bock et al., 2004).

Although injury caused by the feeding activity of ECB is considered as a predisposing factor for mycotoxin occurrence in maize (Blandino et al., 2015; Williams et al., 2002; Windham et al., 1999), the contamination is actually the result of the combination of a number of factors, including also environmental, climatic and agronomic variables (Abbas et al., 2009; Blandino et al., 2015). Hence, when insect damages occurred simultaneously with favourable climatic conditions, high mycotoxin concentrations were usually found in maize grains. In our study, a positive correlation between the mean number of larvae and pupae of ECB and AFB_1 concentration was observed only in a predisposing season like 2017, in a site characterized by a severe infestation

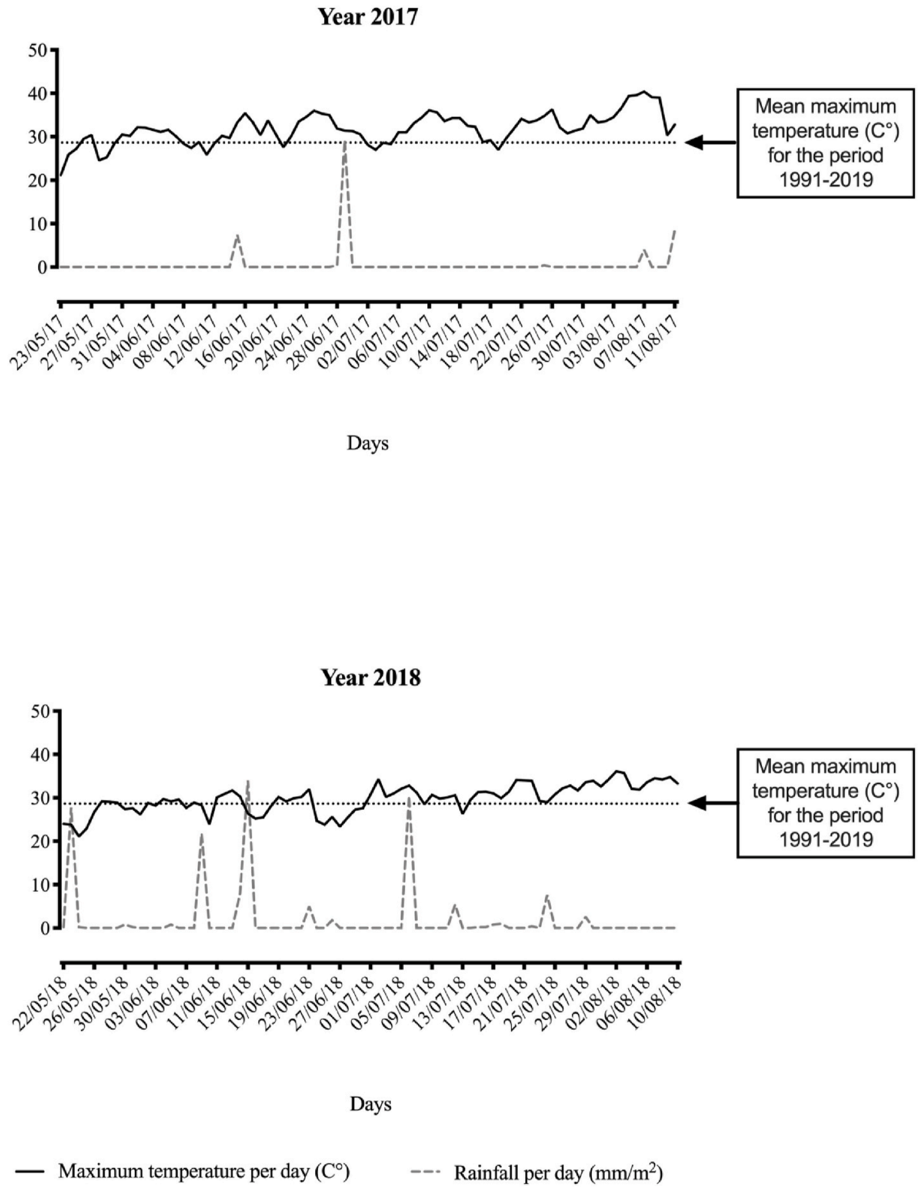

Fig. 8. Seasonal trend of daily maximum temperature (°C) and rainfall (mm/m^2) in the period from 22nd of May to 10th of August in both sampling seasons.

supporting the predominant role of meteorological conditions over ECB infestations on AFB_1 production in pre-harvest maize.

5. Conclusions

A practical contribution for the sustainable maize production in Italy was here reported, demonstrating that biological control strategy, although less effective than chemical control on suppressing ECB infestations, can be feasible in biological system to reduce the second larval generation, without any yield loss and increment of AFB_1 in kernels.

Strategies focused on the reduction of aflatoxin contamination in pre-harvest maize will be a new challenge in this field for the next future, especially considering the ongoing climate change scenario. In a recent paper, Battilani et al. (2016) showed that an increase of 2 °C, which is currently considered as the most reliable forecast scenario, for the next 100 years (Tollefson, 2015), could strongly influence the aflatoxin contamination in maize kernels in Europe (Battilani et al., 2016) highlighting the need for new research on this topic.

Credit authorship contribution statement

Serena Magagnoli: Investigation, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Alberto Lanzoni:** Investigation, Methodology, Data curation, Writing - review & editing. **Antonio Masetti:** Data curation, Formal analysis, Writing - review & editing. **Laura Depalo:** Investigation, Writing - review & editing. **Marco Albertini:** Resources, Conceptualization, Methodology, Writing - review & editing. **Roberto Ferrari:** Conceptualization, Investigation, Writing - review & editing. **Giorgio Spadola:** Investigation, Reviewing. **Francesca Degola:** Investigation, Writing - review & editing. **Francesco M. Restivo:** Funding acquisitition, Project administration, Investigation, Writing - review & editing. **Giovanni Burgio:** Funding acquisition, Project administration, Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

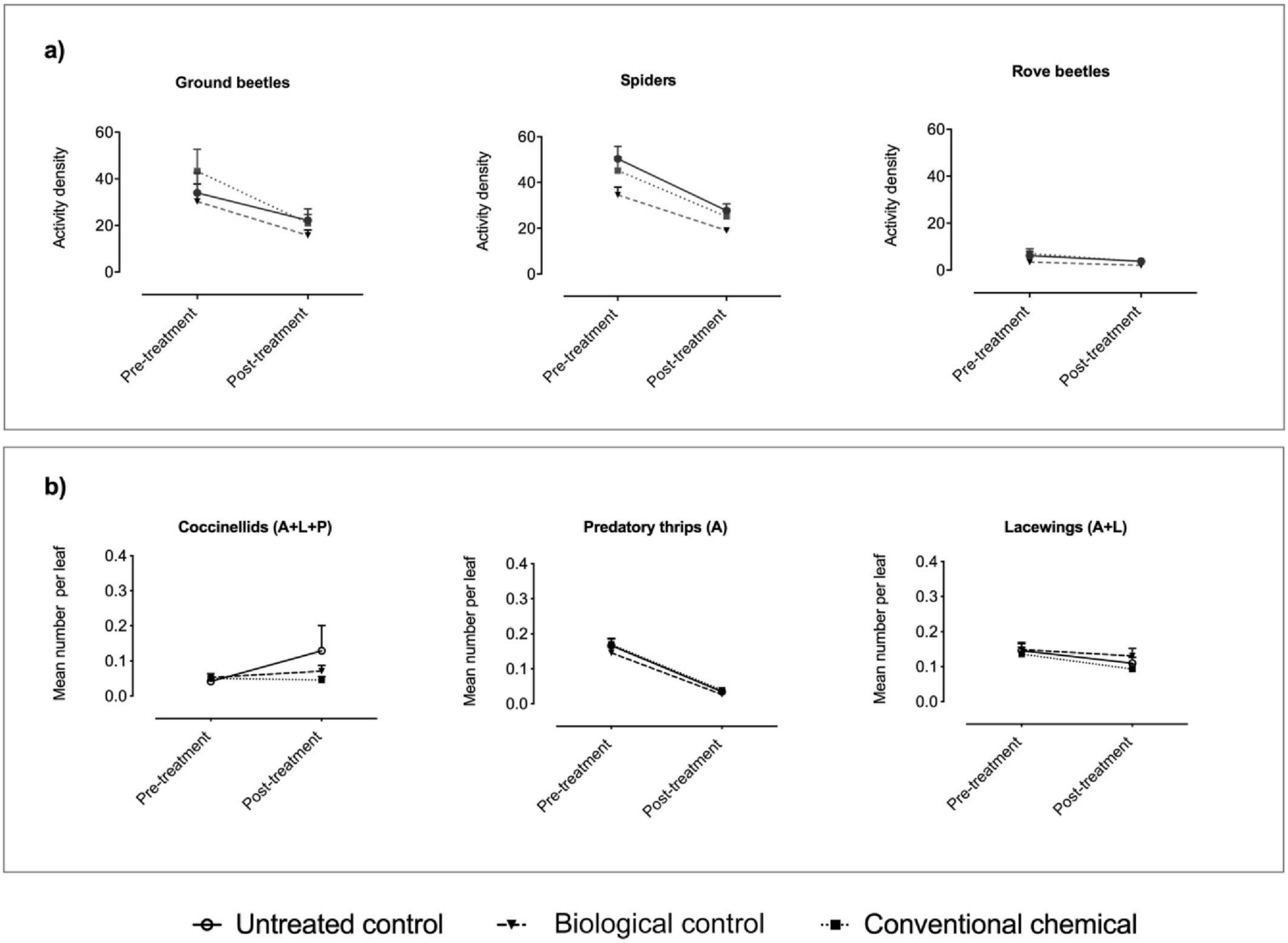

Fig. 9. **a)** The activity density (+SE) of ground-dwelling arthropods (ground beetles, rove beetles and spiders) and **b)** the mean number per leaf (+SE) of the more abundant beneficial insects (coccinellids, predatory thrips and lacewings) were monitored pre- and post-treatment in each strategy (pooled data of sites and years). No significant differences were found among strategies. A = adults, L = larvae, P = pupae.

the work reported in this paper.

Acknowledgments

This work was funded by PSR Emilia-Romagna 2014–2020 Mis. 16.1.01 (Focus Area 4B; PSR DIFESAMAIS number 5004325), European Fund for Rural Development. We are indebted with Riccardo Nicoli, Giorgio Dalle Molle, Giacomo Marani, Giacomo Poluzzi, Orlando Vezzoli and Elisa Tarantino for the field assistance in the project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2020.105529.

References

Abbas, H., Wilkinson, J., Zablotowicz, R., Accinelli, C., Abel, C., Bruns, H., Weaver, M., 2009. Ecology of Aspergillus flavus, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. Toxin Rev. 28, 142–153.

Alma, A., Lessio, F., Reyneri, A., Blandino, M., 2005. Relationships between Ostrinia nubilalis (Lepidoptera: Crambidae) feeding activity, crop technique and mycotoxin contamination of corn kernel in northwestern Italy. Int. J. Pest Manag. 51, 165–173.

Battilani, P., Toscano, P., Van der Fels-Klerx, H., Moretti, A., Camardo Leggieri, C., Brera, C., Rortais, A., Goumperis, T., Robinson, T., 2016. Aflatoxin B 1 contamination in maize in Europe increases due to climate change. Sci. Rep. 6, 24328.

Bigler, F., 1986. Mass production of Trichogramma maidis Pint, et Voeg. and its field application against Ostrinia nubilalis Hbn. in Switzerland 1. J. Appl. Entomol. 101, 23–29.

Blandino, M., Scarpino, V., Vanara, F., Sulyok, M., Krska, R., Reyneri, A., 2015. Role of the European corn borer (Ostrinia nubilalis) on contamination of maize with 13 Fusarium mycotoxins. Food Addit. Contam. 32, 533–543.

Bock, C., Mackey, B., Cotty, P., 2004. Population dynamics of Aspergillus flavus in the air of an intensively cultivated region of south-west Arizona. Plant Pathol. 53, 422–433.

Bravo, A., Likitvivatanavong, S., Gill, S.S., Soberón, M., 2011. Bacillus thuringiensis: a story of a successful bioinsecticide. Insect Biochem. Mol. Biol. 41, 423–431.

Brugger, K.E., Cole, P.G., Newman, I.C., Parker, N., Scholz, B., Suvagia, P., Walker, G., Hammond, T.G., 2010. Selectivity of chlorantraniliprole to parasitoid wasps. Pest Manag. Sci. 66, 1075–1081.

Burgio, G., Maini, S., 1995. Control of European corn borer in sweet corn by Trichogramma brassicae Bezd.(Hym., Trichogrammatidae). J. Appl. Entomol. 119, 83–87.

Camardo Leggieri, M., Bertuzzi, T., Pietri, A., Battilani, P., 2015. Mycotoxin occurrence in maize produced in Northern Italy over the years 2009–2011: focus on the role of crop related factors. Phytopathol. Mediterr. 54, 212–221.

Camerini, G., Groppali, R., Rama, F., Maini, S., 2015. Semiochemicals of Ostrinia nubilalis: diel response to sex pheromone and phenylacetaldehyde in open field. Bull. Insectol. 68, 45–50.

Cheli, F., Battaglia, D., Gallo, R., Dell'Orto, V., 2014. EU legislation on cereal safety: an update with a focus on mycotoxins. Food Contr. 37, 315–325.

Chen, Z.-Y., Brown, R.L., Cleveland, T.E., 2004. Evidence for an association in corn between stress tolerance and resistance to Aspergillus flavus infection and aflatoxin contamination. Afr. J. Biotechnol. 3, 693–699.

Coppock, R.W., Christian, R.G., Jacobsen, B.J., 2018. Aflatoxins. In: Veterinary Toxicology, third ed. Elsevier, pp. 983–994.

Cotty, P.J., Jaime-Garcia, R., 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int. J. Food Microbiol. 119, 109–115.

Depalo, L., Lanzoni, A., Masetti, A., Pasqualini, E., Burgio, G., 2017. Lethal and sub-lethal effects of four insecticides on the aphidophagous coccinellid Adalia bipunctata (Coleoptera: Coccinellidae). J. Econ. Entomol. 110, 2662–2671.
Dinter, A., Brugger, K.E., Frost, N.-M., Woodward, M.D., 2010. Chlorantraniliprole (Rynaxypyr): a novel DuPont^{TM} insecticide with low toxicity and low risk for honey bees (Apis mellifera) and bumble bees (Bombus terrestris) providing excellent tools for uses in integrated pest management. Julius-Kühn-Arch. 84.
Esmaeilishirazifard, E., Keshavarz, T., 2014. Aflatoxin occurrence. Aflatoxins-Food Sources Occur. Toxicol. Eff. Ed AG Faulkner Nova Publ. N. Y. USA 35–63.
European Commission, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. J Eur Union 364.
FAOSTAT, F., 2016. FAOSTAT Statistical Database.
Fernández-Ibañez, V., Soldado, A., Martínez-Fernández, A., De la Roza-Delgado, B., 2009. Application of near infrared spectroscopy for rapid detection of aflatoxin B1 in maize and barley as analytical quality assessment. Food Chem. 113, 629–634.
Gontijo, P.C., Moscardini, V.F., Michaud, J., Carvalho, G.A., 2014. Non-target effects of chlorantraniliprole and thiamethoxam on Chrysoperla carnea when employed as sunflower seed treatments. J. Pest. Sci. 87, 711–719.
Guo, B., Chen, Z., Lee, R.D., Scully, B.T., 2008. Drought stress and preharvest aflatoxin contamination in agricultural commodity: genetics, genomics and proteomics. J. Integr. Plant Biol. 50, 1281–1291.
Hassan, S., Langenbruch, G., Neuffer, G., 1978. The influence of the hosts used for mass-rearing on the quality of the egg parasite Trichogramma evanescens in relation to the control of the maize borer, Ostrinia nubilalis. Entomophaga 23, 321–329.
Hwang, I.-C., Park, C., Kang, D.-K., Jin, N.-Y., Jung, S., Seo, M.-J., Kim, J.-E., Youn, Y.-N., Yu, Y.-M., 2010. Combined application of Trichogramma ostriniae and Bacillus thuringiensis for eco-friendly control of Plutella xylostella. J. Korean Soc. Appl. Biol. Chem. 53, 316–322.
Klich, M.A., 2007. Aspergillus flavus: the major producer of aflatoxin. Mol. Plant Pathol. 8, 713–722.
Leff, B., Ramankutty, N., Foley, J.A., 2004. Geographic distribution of major crops across the world. Global Biogeochem. Cycles 18.
Magagnoli, S., Masetti, A., Depalo, L., Sommaggio, D., Campanelli, G., Leteo, F., Lövei, G. L., Burgio, G., 2018. Cover crop termination techniques affect ground predation within an organic vegetable rotation system: a test with artificial caterpillars. Biol. Contr. 117, 109–114.
Maini, S., Burgio, G., 1999. Ostrinia nubilalis (Hb.)(Lep., Pyralidae) on sweet corn: relationship between adults caught in multibaited traps and ear damages. J. Appl. Entomol. 123, 179–185.
Medina, A., Rodriguez, A., Magan, N., 2014. Effect of climate change on Aspergillus flavus and aflatoxin B1 production. Front. Microbiol. 5, 348.
Meissle, M., Mouron, P., Musa, T., Bigler, F., Pons, X., Vasileiadis, V., Otto, S., Antichi, D., Kiss, J., Pálinkás, Z., 2010. Pests, pesticide use and alternative options in European maize production: current status and future prospects. J. Appl. Entomol. 134, 357–375.
Oatman, E., Wyman, J., Van Steenwyk, R., Johnson, M., 1983. Integrated control of the tomato fruitworm (Lepidoptera: Noctuidae) and other lepidopterous pests on fresh-market tomatoes in southern California. J. Econ. Entomol. 76, 1363–1369.
Payne, G., Brown, M., 1998. Genetics and physiology of aflatoxin biosynthesis. Annu. Rev. Phytopathol. 36, 329–362.
Payne, G.A., Widstrom, N.W., 1992. Aflatoxin in maize. Crit. Rev. Plant Sci. 10, 423–440.
Pimentel, D., 2006. Soil erosion: a food and environmental threat. Environ. Dev. Sustain. 8, 119–137.
Preetha, G., Stanley, J., Suresh, S., Kuttalam, S., Samiyappan, R., 2009. Toxicity of selected insecticides to Trichogramma chilonis: assessing their safety in the rice ecosystem. Phytoparasitica 37, 209–215.
Rainio, J., Niemelä, J., 2003. Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodivers. Conserv. 12, 487–506.
Razinger, J., Vasileiadis, V.P., Giraud, M., van Dijk, W., Modic, Š., Sattin, M., Urek, G., 2016. On-farm evaluation of inundative biological control of Ostrinia nubilalis (Lepidoptera: Crambidae) by Trichogramma brassicae (Hymenoptera: Trichogrammatidae) in three European maize-producing regions. Pest Manag. Sci. 72, 246–254.
Sanchis, V., 2011. From microbial sprays to insect-resistant transgenic plants: history of the biospesticide Bacillus thuringiensis. A review. Agron. Sustain. Dev. 31, 217–231.
Sanchis, V., Magan, N., 2004. Environmental conditions affecting mycotoxins. Mycotoxins Food Detect. Contr. 174–189.
Smagghe, G., Deknopper, J., Meeus, I., Mommaerts, V., 2013. Dietary chlorantraniliprole suppresses reproduction in worker bumblebees. Pest Manag. Sci. 69, 787–791.
Suverkropp, B.P., Bigler, F., van Lenteren, J.C., 2009. Dispersal behaviour of Trichogramma brassicae in maize fields. Bull. Insectol. 62, 113–120.
Tilman, D., 1999. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proc. Natl. Acad. Sci. Unit. States Am. 96, 5995–6000.
Tilman, D., 1998. The greening of the green revolution. Nature 396, 211–212.
Tollefson, J., 2015. Global-warming limit of 2 C hangs in the balance. Nature 520, 14–15.
Tóth, M., Szarukán, I., Nagy, A., Ábri, T., Katona, V., Kőrösi, S., Nagy, T., Szarvas, Á., Koczor, S., 2016. An improved female-targeted semiochemical lure for the European corn borer Ostrinia nubilalis Hbn. Acta Phytopathol. Entomol. Hung. 51, 247–254.
Umesha, S., Manukumar, H.M., Chandrasekhar, B., Shivakumara, P., Shiva Kumar, J., Raghava, S., Avinash, P., Shirin, M., Bharathi, T.R., Rajini, S.B., Nandhini, M., Rani, G.V., Shobba, M., Prakash, H.S., 2017. Aflatoxins and food pathogens: impact of biologically active aflatoxins and their control strategies. J. Sci. Food Agric. 97, 1698–1707.
Vasileiadis, V., Sattin, M., Otto, S., Veres, A., Pálinkás, Z., Ban, R., Pons, X., Kudsk, P., Van Der Weide, R., Czembor, E., 2011. Crop protection in European maize-based cropping systems: current practices and recommendations for innovative Integrated Pest Management. Agric. Syst. 104, 533–540.
Vasileiadis, V.P., Veres, A., Loddo, D., Masin, R., Sattin, M., Furlan, L., 2017. Careful choice of insecticides in integrated pest management strategies against Ostrinia nubilalis (Hübner) in maize conserves Orius spp. in the field. Crop Protect. 97, 45–51.
Voegele, J., 1975. The genus Trichogramma, microhymenopterae. Presented at the Semaine d'Etude Agriculture et Hygiene des Plantes, Gembloux (Belgium), 8 Sep 1975, Faculte des Sciences Agronomiques de l'Etat et Centre de Recherches Agronomiques.
Warburton, M.L., Wilkes, G., Taba, S., Charcosset, A., Mir, C., Dumas, F., Madur, D., Dreisigacker, S., Bedoya, C., Prasanna, B., 2011. Gene flow among different teosinte taxa and into the domesticated maize gene pool. Genet. Resour. Crop Evol. 58, 1243–1261.
Widstrom, N., 1979. The role of insects and other plant pests in aflatoxin contamination of corn, cotton, and peanuts—a review 1. J. Environ. Qual. 8, 5–11.
Williams, W.P., Buckley, P.M., Windham, G.L., 2002. Southwestern corn borer (Lepidoptera: Crambidae) damage and aflatoxin accumulation in maize. J. Econ. Entomol. 95, 1049–1053.
Windham, G., Williams, W., Davis, F., 1999. Effects of the southwestern corn borer on Aspergillus flavus kernel infection and aflatoxin accumulation in maize hybrids. Plant Dis. 83, 535–540.

Web references

(last access on November 18, 2020). https://simc.arpae.it/dext3r/.
(last access on November 18, 2020). https://www.arpae.it.