Research Article

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On-farm evaluation of inundative biological control of *Ostrinia nubilalis* (Lepidoptera: Crambidae) by *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) in three European maize-producing regions

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Abstract

BACKGROUND: A 2 year study was conducted to evaluate the efficacy of biological control with optimally timed *Trichogramma brassicae* releases as an integrated pest management tool against the European corn borer (ECB), *Ostrinia nubilalis* (Hübner), in on-farm experiments (i.e. real field conditions) in three European regions with dissimilar geoclimatic conditions and ECB pressure and conventional management (i.e. insecticide treated and untreated).

RESULTS: Biological control with *Trichogramma* (1) provided ECB protection comparable with conventional management, (2) in all cases maintained mycotoxin levels below the EU threshold for maize raw materials destined for food products, (3) was economically sustainable in southern France and northern Italy, but not in Slovenia where it resulted in a significant decrease in gross margin, mainly owing to the cost of *Trichogramma* product, and (4) enabled avoidance of detrimental environmental effects of lambda-cyhalothrin use in northern Italy.

CONCLUSION: Optimally timed mass release of *T. brassicae* could be considered a sustainable tool for IPM programmes against ECB in southern France and northern Italy. Better involvement of regional advisory services is needed for the successful dissemination and implementation of biological control. Subsidy schemes could also motivate farmers to adopt this IPM tool and compensate for high costs of *Trichogramma* product.

Keywords: European corn borer; biological control; integrated pest management; mycotoxins; pesticide risk reduction; economic sustainability

1 INTRODUCTION

Maize (*Zea mays* L.) is one of the most important crops in Europe, covering an area of approximately 19 million ha, with an estimated production of 117 million t in 2013.[1] Grain maize production dominates in central and southern Europe, while maize is typically grown for silage in northern Europe.[2] It is grown either as monoculture or in rotation with other crops, and crop protection is mainly pesticide based with different levels of IPM implementation.[3] In spite of the differences in maize cropping, a common set of weeds, arthropod pests and fungal diseases is responsible for the main problems in most European regions.[2]

Corn borers represent the main biotic stressor for maize crops in Europe.[2] The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is the most important maize pest in most parts of Europe, while the Mediterranean corn borer (MCB), *Sesamia nonagrioides* Lef., is predominant in warmer areas of southern Europe.[2,4–6] ECB damage is mostly caused by larvae that enter the maize stalk after hatching and feed on the pith. Yield is affected by ECB damage to

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Correction Note: This article was first published online on the 2nd of July 2015. It was corrected on the 7th of September 2015. For further clarification, the G1, G2 and G3 in the caption of Figure 2 indicate: G1, first adult flight, beginning of first generation; G2, second adult flight, beginning of second generation; G3, third adult flight, beginning of third generation. Readers are also encouraged to read the paper 'Control of European corn borer in sweet corn by Trichogramma brassicae *Bezd. (Hym., Trichogrammatidae)' by G. Burgio and S. Maini,* Journal of Applied Entomology, *119, 83–87, 1995, and references therein, for information about* Trichogramma *experiments performed in Italy.*

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the ear and tunnelling in the stalk, resulting in breakage. The latter is more important regarding yield loss.[7–9] In addition, ECB damage can affect plant health by vectoring *Fusarium moniliforme* and facilitating fungal infections.[8] ECB damage on ears affects grain quality and has been described as an important factor for favouring high levels of fumonisins in maize kernels.[8,9]

Foliar insecticide applications are conventionally used on maize in many European countries (e.g. Spain, Hungary, Poland, Germany, Italy, France and Denmark), with different frequency and percentage of crop area treated. The main target of spray insecticides are corn borers, but applications against western corn rootworm (*Diabrotica virgifera virgifera* LeConte) adults and corn earworm (*Helicoverpa armigera* Hübner) larvae have also been reported.[2] However, to meet the worldwide food demand, as well as to satisfy growing environmental concerns, farmers must increase food production and quality while reducing its detrimental ecological impact, and this is a major challenge.[10–12] The reduction in dependence on pesticides, as well as the risks and adverse impacts of their use on human health and the environment, is an integral part of the European Union's agenda for agriculture (Directive 2009/128/EC).[13] A key element for reaching this goal is to promote the implementation of integrated pest management (IPM), which has been compulsory in the EU since the beginning of 2014. Several alternatives have been suggested to reduce the dependence on insecticides for ECB control in maize crops, for example the breeding of maize cultivars with increased tolerance to insects,[3,4,14] the use of pheromone mating disruption,[15,16] and the use of *Bt* maize expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis*.[17] However, owing to the rather conservative EU policies on genetically modified crops,[12] in concert with the strong opposition of European consumers both to their cultivation and to the extensive application of pesticides,[9] a more acceptable way to control ECB, in line with the Directive 2009/128/EC, is by means of biological control, which was the focus of the present study.

Mass release of *Trichogramma* spp. is a promising alternative for ECB control in the EU, avoiding, or at least reducing, the need for broad-spectrum insecticide applications. The parasitoid wasps are being released against ECB on about 150 000 ha of maize per year, mainly in France, Germany and Switzerland, with the widest implementation in France.[2] Several studies have been performed worldwide, the majority in the United States, with an overall positive evaluation of the efficacy of mass release of *Trichogramma* spp. against corn borers in maize, alone or in combination with insecticide applications.[18–26] However, to our knowledge, apart from some country-specific studies, robust evidence of the sustainability of *Trichogramma* spp. on a European-wide scale is lacking, and consequently so is the motivation of stakeholders to adopt and implement biological control against ECB as a sustainable tool for IPM systems in countries where release of *Trichogramma* spp. is not currently being used. This can only be achieved by assessing and validating biological control using *Trichogramma* spp. at real farm scale under the diverse climatic conditions that represent the reality of European agriculture.

In order to address this, a major working group within the European Project PURE (Pesticide Use-and-Risk reduction in European farming systems with integrated pest management, http://www.pure-ipm.eu) was devoted to evaluation of various IPM tools for a less pesticide-dependent European maize production. A detailed study evaluating various integrated weed management tools in different agroenvironments in Europe was published recently as the first outcome of this working group.[27]

This paper presents the results of the evaluation of inundative *Trichogramma* release as an IPM tool for ECB management. Our aims were (i) to assess the efficacy of optimally timed release of *Trichogramma brassicae* to control ECB in on-farm experiments (i.e. real field conditions on commercial or demonstration farms) against the conventional approach used (i.e. insecticide treated or untreated) in three European grain-maize-producing regions with dissimilar geoclimatic conditions as well as ECB pressure, and (ii) to perform a comparative assessment of its economic sustainability for further recommendation of this IPM tool.

2 EXPERIMENTAL METHODS

2.1 Experimental sites, design and crop management

Nine on-farm experiments were conducted in 2011 and 2012 to compare the efficacy of biological control of ECB using *T. brassicae* against the conventional (CON) management (i.e. insecticide treated or untreated). Three European grain-maize-producing regions were selected for these experiments with different climatic and edaphic conditions as well as types of maize cropping system (see Fig. 1 for a schematic map of the experiments performed in 2011 and 2012). Northern Italy (five farms) represented the southern maize cropping conditions, where the average characteristics are relatively cold winters and warm-hot summers, medium-high water availability (medium-high rainfall or irrigation) and high grain yield potential (>12 t ha^{-1}). Southern France (two farms) represented the western maize cropping conditions, where the average characteristics are mild winters and warm-hot summers, medium-high rainfall and high grain yield potential (>12 t ha^{-1}). Finally, Slovenia (two farms) represented the central-eastern maize cropping conditions, with mild continental climate, medium rainfall during the maize-growing season, no available irrigation and a medium grain yield potential (<12 t ha^{-1}). In each country, a minimum of two farms were used as replicates each year. The farm selection was based on the farm (a) being located in a representative maize-growing region within the country and (b) following the conventional maize production typical for that country. The experimental plots within the farms were chosen randomly. Two plots of the same size (0.5–1 ha) were created on each farm, with one plot being managed with the CON approach (i.e. insecticide treated or untreated, depending on normal practices in that specific country) and the other using biological control with *T. brassicae* (TRI) as the IPM tool against ECB. As all farms implemented crop rotation, CON and TRI plot locations differed each year on every farm, were never close to field margins and were at least 100 m apart to avoid dispersal of *Trichogramma* to CON plots. In order to be able to separate the effects of the IPM tool from the conventional approach on the maize grain yields, the same crop and weed management was applied to the two plots on each farm, and thus they differed only in ECB management. All on-farm experiments were managed with commercially available or technologically mature equipment suited to field-scale applications.

2.2 On-farm releases of *Trichogramma*

In southern France there is a long tradition of *Trichogramma* release against ECB. Therefore, only one parasitoid release was performed. In contrast, in Italy and Slovenia, owing to limited experience[20] with *Trichogramma* release, two releases were performed at a 2 week interval to ensure that the egg laying period was properly covered. The wasps were released at a density of 375 000 *Trichogramma* wasps ha^{-1} from a total of 50 dispensers

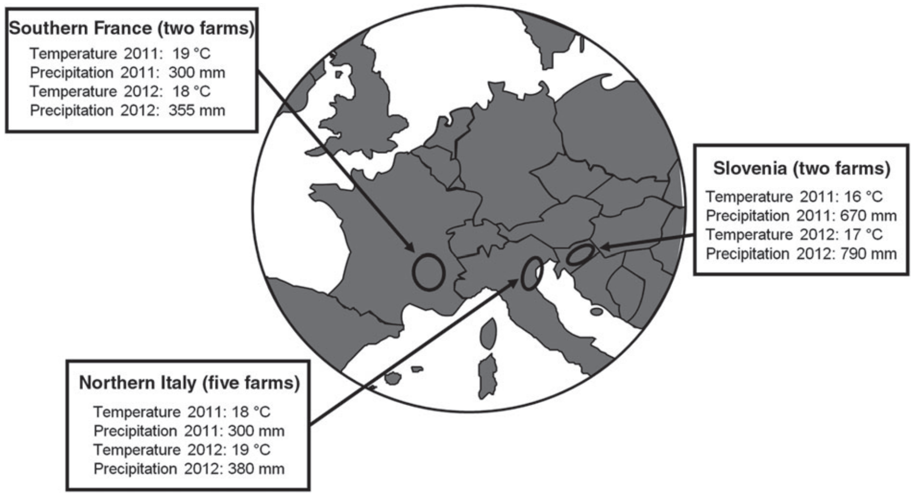

Figure 1. Map of experimental locations per country, showing the average temperature (°C) and total precipitation (mm) for each growing season (April to October) (after Meissle *et al.*[2]).

Table 1. *Ostrinia nubilalis* management in different countries

Country	Year	Plot	ECB management[a]	Dates of treatment
France (two farms)	2011	CON	No treatment	No treatment
		TRI	1 × 375 000 *T. brassicae* ha^{-1}	7/7
	2012	CON	No treatment	No treatment
		TRI	1 × 375 000 *T. brassicae* ha^{-1}	17/7
Italy (five farms)	2011	CON[b]	Lambda-cyhalothrin, Syngenta; 25 g ha^{-1}	12/7, 16/7, 20/7, 21/7, 22/7
		TRI	2 × 375 000 *T. brassicae* ha^{-1}	6/7, 20/7
	2012	CON[b]	Lambda-cyhalothrin, Syngenta; 25 g ha^{-1}	16/7, 18/7, 19/7, 19/7, 9/8
		TRI	2 × 375 000 *T. brassicae* ha^{-1}	12/7, 25/7
Slovenia (two farms)	2011	CON	No treatment	No treatment
		TRI	2 × 375 000 *T. brassicae* ha^{-1}	8/7, 20/7
	2012	CON	No treatment	No treatment
		TRI	2 × 375 000 *T. brassicae* ha^{-1}	25/7, 7/8

[a] Insecticide rate is expressed as mass AI ha^{-1}.
[b] There was only one insecticide application per farm in Italy.

ha^{-1}, as is the standard in Switzerland.[28] The *T. brassicae* were supplied by Biotop (Livron-sur-Drôme, France). In Italy, insecticides were applied to the CON plots (i.e. normal agricultural practice). In order to prevent spray drift to the TRI plots, fields between and around experimental plots were grown with winter wheat in Italy. In France and Slovenia there was no insecticide application on CON plots. Details of the different ECB management strategies per country are given in Table 1.

2.3 Timing of *T. brassicae* releases and parasitism assessment

T. brassicae releases were performed against the second ECB generation in all countries, as it is more damaging to the crop.[2,5] The release date was fine-tuned on the basis of the monitoring of ECB flight dynamics by light traps in each country and observation of their pupation dynamics. When the first ECB flight was waning, the evaluation of pupation was initiated: ECB-infested maize plants were cut, and ECB larvae and pupae on leaves and inside stalks were counted. To obtain reliable pupation results, enough infested plants were sampled to obtain at least 30 living individuals (total number of larvae and pupae). *Trichogramma* were released 1 week after the threshold range of 25–30% of pupation was reached. This information was sent to Biotop for them to reactivate the *T. brassicae* and prepare the product. In Italy and Slovenia, a second release was performed approximately 2 weeks after the first one (Table 1).

Second-generation ECB pressure was assessed in both CON and TRI plots by scouting for egg masses on the abaxial side of maize leaves on 100 plants per plot. *T. brassicae* parasitisation was assessed by categorising egg masses found in the TRI plots as 'fresh' (F), 'parasitised' (P) or 'hatching' (H). Scouting was done twice during the second-generation egg laying period, 10 and 20 days after the first *Trichogramma* release. Based on these observations, the *T. brassicae* apparent parasitism rate was calculated using the following equation: $P\,(\%) = P/(F + P + H)$.

2.4 *Ostrinia nubilalis* damage and *Fusarium* spp. presence assessments

Two ECB damage assessments were made on CON and TRI plots. First-generation ECB damage was assessed by visual examination of leaves and stalks of 100 plants per plot just prior to second-generation flight. The second assessment was performed before harvest in two sampling areas of 20 m × 6 maize rows per plot, where the total number of plants (final maize stand), plants broken above ear, plants broken below ear and plants with any symptoms of ECB attack (e.g. holes in leaves, stalks or cobs) were recorded. In addition, ECB damage and *Fusarium* spp. presence on ears was observed on ten plants from each sampling area. The levesl of ECB damage and *Fusarium* spp. presence on ears were assessed using a scale from 1 to 7, where 1 = no ECB damage or no *Fusarium* spp., 2 = <4%, 3 = 5 – 10%, 4 = 11 – 25%, 5 = 25 – 50%, 6 = 50 – 75%7 and 7 = >75% surface damaged by ECB or colonised by *Fusarium* spp. Final ECB damage assessments on maize plants and ears and *Fusarium* spp. presence on ears were not recorded in Slovenia in 2012.

2.5 Yield and mycotoxin assessments

Yield was assessed by harvesting the whole CON and TRI plots using a combine harvester. Grain moisture was determined by standardised methods (ISO 711:1997[29]), and grain yield was then expressed in t ha^{-1} at 14% moisture content.

During harvesting, a 2 – 3 kg grain yield sample was taken from each plot for mycotoxin analysis by collecting multiple small grain samples at successive moments when grain was discharged from the combine harvester. These samples were then placed in a freezer (at −18 °C) prior to transportation to a certified laboratory for analysis of the fumonisin B1 and B2 levels in grain.

2.6 Cost-benefit analysis of conventional management versus biological control

A template was developed and provided to all partners for data collection of the crop management costs (costs of inputs, e.g. fertilisers, pesticides and *Trichogramma* product; costs of operations, e.g. pesticide spraying, labour costs and fuel) and crop yields. For costs of inputs, the prices that farmers paid were used, whereas the operation costs were based on contract work prices, including costs for labour, machinery and fuel, provided by regional contracting companies. For manual work (e.g. application of *Trichogramma*), labour prices were derived from the Farm Accountancy Data Network. Grain maize prices for 2011 and 2012 were obtained from the Eurostat database. Crop yields, prices and costs of inputs and operations (in euros) were used for calculation of CON and TRI gross margins: gross margin = financial yield (yield × price) − variable costs].[30]

2.7 Environmental risk

The environmental risk related to the insecticide used for ECB control in the CON treatment in Italy was assessed with the model SYNOPS.[31] The model calculates the acute and chronic risks of pesticides for aquatic (reference species: *Daphnia* sp., fish, algae, *Lemna* sp. and *Chironomus* sp.) as well as terrestrial organisms (reference species: earthworms and bees), based on site characteristics and application data. The risks are expressed as the exposure toxicity atio (ETR). For low environmental risks, ETR values should be <0.1 for acute risk and <1.0 for chronic risk.

2.8 Statistical analyses

All statistical analyses were performed with Statistica 10 (StatSoft Inc., 2011). All raw data were checked for normality of distribution using the Kolmogorov – Smirnov test (K – S) prior to statistical analysis. Data of first-generation (G1) damage and second-generation (G2) egg masses per country/region were normally distributed, and analysis of variance (ANOVA), with plots (i.e. CON and TRI) and years as main factors, was performed to identify differences between plots per year in damage and pressure before any treatments. In order to investigate the year effect and differences among countries on *Trichogramma* parasitism in TRI plots, proportional data were first arcsine square root transformed and then subjected to ANOVA with country and year as main factors. Proportional data recorded from ECB damage on final maize stand were also arcsine square root transformed, whereas data from ECB damage and *Fusarium* spp. presence on ears and mycotoxin contamination were normally distributed. One-way ANOVA was used for these parameters to identify significant differences between insecticide-treated versus TRI (in Italy) and untreated versus TRI (in France and Slovenia) respectively. Data on grain yield, total costs and gross margin per country/region did not require any transformation and were analysed by factorial ANOVA with year and ECB management as main factors respectively. Means obtained by ANOVA were compared using Fisher's protected LSD test at $P = 0.05$.

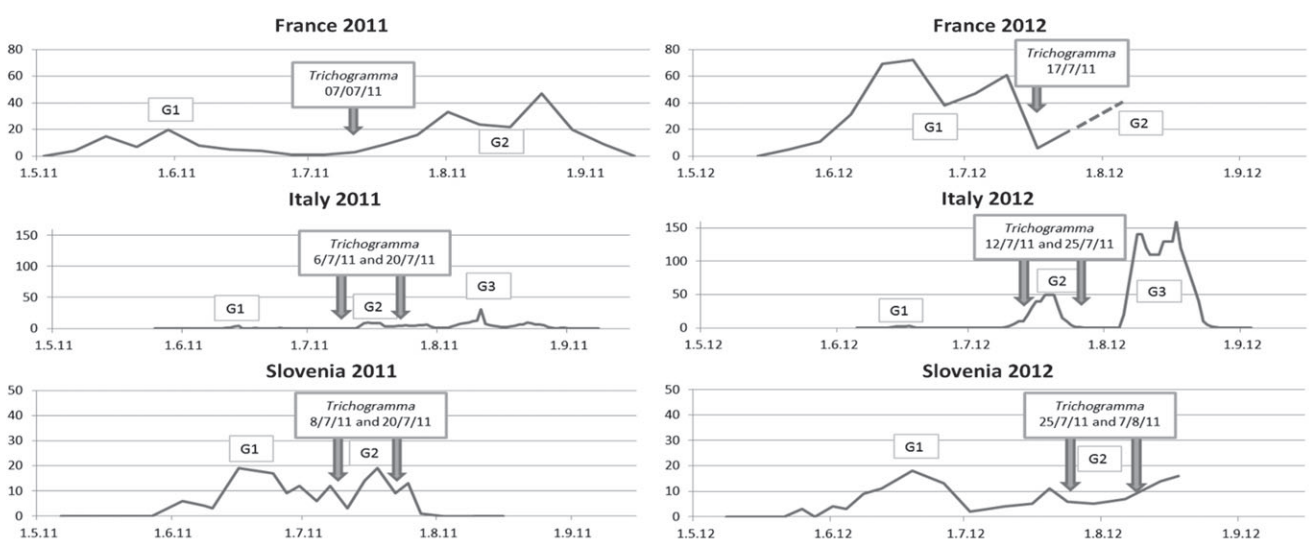

Figure 2. *Ostrinia nubilalis* monitoring with light traps and *Trichogramma brassicae* release in the different countries during the 2 year study. G1, first adult flight, beginning of first generation; G2, second adult flight, beginning of second generation; G3, third adult flight, beginning of third generation.

Table 2. *Ostrinia nubilalis* damage from first-generation (G1) and second-generation (G2) egg masses in conventional (CON) and *Trichogramma* (TRI) plots of each country before any treatments. Data presented are means of several farms for each country ± standard error

Country/region	Year	Plots	Plant damage from G1 (%)	Egg masses from G2 (from 100 plants)
Southern France	2011	CON	4.00 ± 1.00	2.00 ± 2.00
		TRI	4.00 ± 1.00	11.25 ± 1.75
	2012	CON	16.00 ± 12.00	19.00 ± 7.00
		TRI	7.00 ± 7.00	12.00 ± 4.00
Northern Italy	2011	CON	14.86 ± 3.43	24.06 ± 14.53
		TRI	6.43 ± 1.49	27.48 ± 9.76
	2012	CON	10.03 ± 5.28	58.50 ± 36.77
		TRI	13.30 ± 5.72	53.30 ± 31.90
Slovenia	2011	CON	3.25 ± 0.25	3.37 ± 1.75
		TRI	5.50 ± 4.50	5.00 ± 3.75
	2012	CON	5.02 ± 3.98	7.50 ± 4.38
		TRI	5.02 ± 3.48	8.44 ± 5.94

Pearson's product moment correlation coefficient (Pearson's r) was performed to identify the relationships between (a) G1 ECB adults caught in light traps and G1 plant damage, (b) G2 egg masses and G2 light trap catches, (c) mycotoxin concentration in grain with ECB damage on ears and *Fusarium* spp. presence on ears and (d) ECB damage on ears and *Fusarium* spp. presence on ears, by pooling data from countries, farms, years and ECB management regime. The coefficient of determination (r^2) was calculated from Pearson's r.

3 RESULTS

The ECB flight dynamics varied strongly between countries. ECB occurred first in France, then in Slovenia and last in Italy (Fig. 2). The highest catches during the first ECB flight were recorded in France, followed by Slovenia and Italy, whereas the highest catches during the second ECB flight were in Italy, followed by France and Slovenia. A third ECB flight was also recorded during both years in Italy.

The percentage of maize plants damaged by G1 differed greatly between countries and years (Table 2). Overall, the lowest G1 damage was reported in Slovenia, and the greatest in Italy (i.e. mean of CON and TRI plots per country, as no treatments had yet been applied). The mean G2 egg masses before any treatments varied considerably within and between countries (Table 2). It ranged from 58.5 egg masses per 100 plants in Italy (2012, CON) to 2.0 egg masses in France (2011, CON plot). ANOVA of G1 damage and G2 egg masses showed no difference between plots and years per country, and thus no variability in ECB damage and pressure among plots prior to treatments. Correlation analysis showed a positive relationship between G1 adult catches by light traps and the percentage of maize plants attacked by G1 ($r^2 = 0.70$, $P < 0.05$), as well as between G2 egg masses and G2 light trap catches ($r^2 = 0.87$, $P < 0.05$).

The mean parasitism rate in the TRI plots after the *Trichogramma* releases, over the 2 years of study, was 80% in Italy, followed by France (71%) and Slovenia (49%) (Fig. 3). Statistical analysis of the year and country effect, and of their interaction (ANOVA of arcsine-square-root-transformed percentages), on the parasitism rate in the TRI plots showed no significant differences.

Statistical analyses (ANOVA of arcsine-square-root-transformed data) showed no significant difference in ECB damage evaluated at harvest (i.e. broken maize plants below or above ear) between TRI and insecticide treated tested in Italy, and between TRI and untreated tested in France and Slovenia (Fig. 4).

Further statistical analysis of parameters evaluated at harvest showed no significant differences between TRI and insecticide treated (Italy) and between TRI and untreated (France and Slovenia) in *Fusarium* spp. presence on ears, ECB damage on ears and mycotoxin concentration. The highest ECB damage on ears was recorded on the TRI plots in Italy, where it ranged from 1 to 3%. In France and Slovenia, on untreated and TRI plots, as well as on CON plots (insecticide treated) in Italy, ECB damage on ears ranged

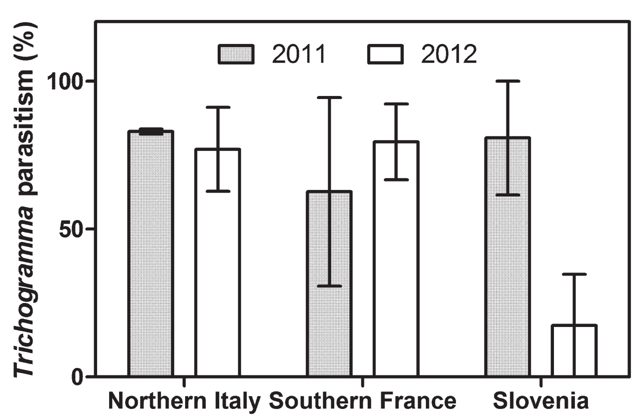

Figure 3. *Trichogramma* parasitism (%) in TRI plots in different countries in 2011 and 2012. Data presented are means of several farms for each country ± standard error.

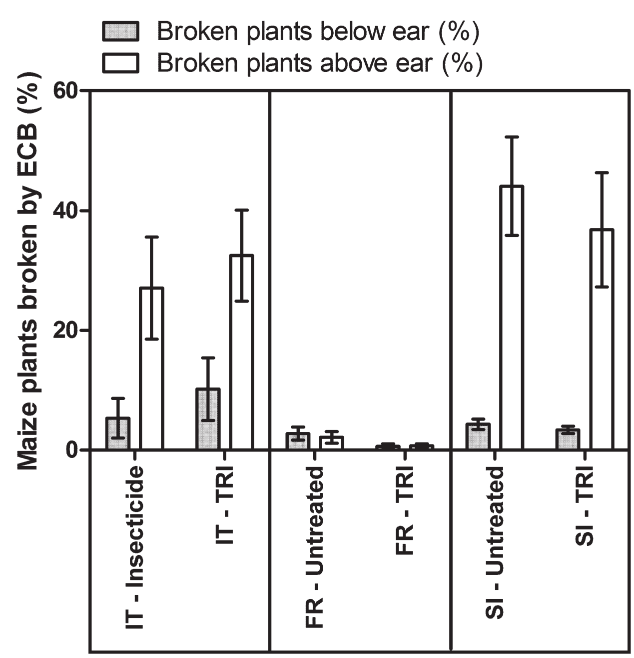

Figure 4. *Ostrinia nubilalis* damage on maize plants (% of broken plants) in different countries and types of ECB management tested (TRI, insecticide treated and untreated), as evaluated at harvest (data presented are means ± standard error for the 2 years and several farms per country). IT, northern Italy; FR, southern France; SI, Slovenia.

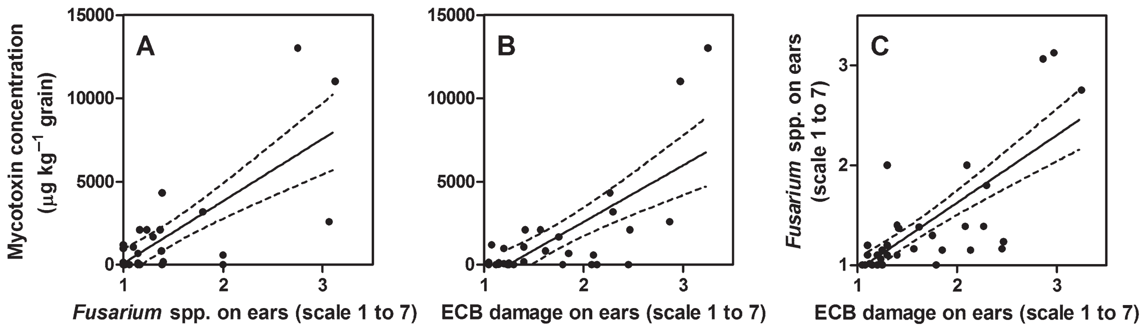

Figure 5. Correlation analysis of mycotoxin concentration in grain with *Fusarium* spp. presence on ears (A) and ECB damage on ears (B) and of *Fusarium* spp. presence on ears with ECB damage on ears (C), as affected by the different ECB management (i.e. *Trichogramma*, insecticide treated and untreated) per country, farm and year. Dots represent the various farms per country and year (see Sections 2.1 and 2.8 for details). Results are presented as raw data and linear regression models (full lines) with 95% confidence intervals (dotted lines).

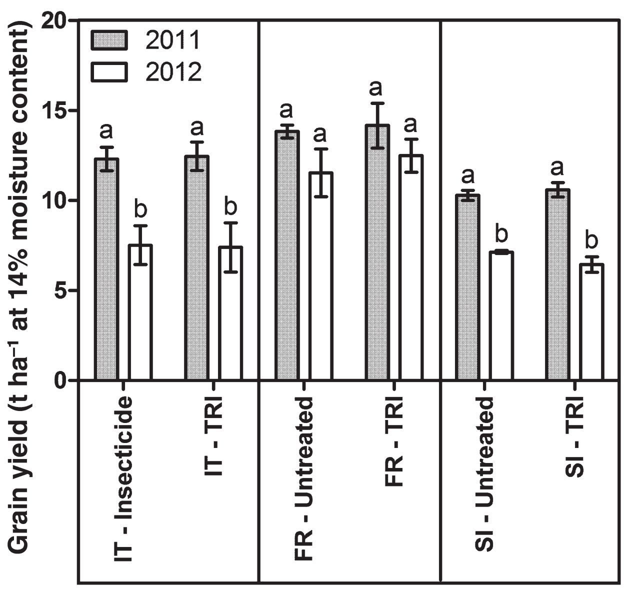

Figure 6. Grain yield per year in different countries and types of ECB management tested (TRI, insecticide treated and untreated). ANOVA was performed on a country/region level. Mean values per country/region not sharing the same lower-case letter are significantly different at $P < 0.05$ according to Fisher's protected LSD test. Data presented are means ± standard error of several farms per country. IT, northern Italy; FR, southern France; SI, Slovenia.

from 0 to 1%. The *Fusarium* spp. presence on ears was 0–1% in all countries and ECB management regimes (not shown).

The mean mycotoxin concentration in grain (i.e. fumonisin B1 and B2) ranged from 2766 µg kg^{-1} grain and 1712 µg kg^{-1} grain in the TRI and CON plots of Italy, respectively, to <605 µg kg^{-1} grain in both other countries and ECB management regimes. Correlation analysis showed a significant relationship of mycotoxin concentration in grain with *Fusarium* spp. presence on ears ($r^2 = 0.56$; $P < 0.0001$) and ECB damage on ears ($r^2 = 0.51$; $P < 0.0001$), as well as of *Fusarium* spp. with ECB damage on ears ($r^2 = 0.60$; $P < 0.0001$) (Figs 5A to C).

ANOVA showed no significant difference in grain yield between ECB management regimes (TRI versus insecticide treated or TRI versus untreated) in all countries. Significant difference between 2011 and 2012 yields was observed in Italy and Slovenia ($P < 0.001$ in both cases), the yields being significantly lower in 2012 owing to adverse weather conditions (Fig. 6).

Compared with the CON management, biological control with *T. brassicae* increased the total variable costs in all countries, ranging on average from + €22 ha^{-1} in Italy (where insecticide treatment was done in the CON plot) to + €107 ha^{-1} in Slovenia (Fig. 7A). Overall, in all countries the variable cost increase was mainly due to the cost of the *Trichogramma* product (+€63 ha^{-1}, +€48 ha^{-1} and + €105 ha^{-1} for Italy, France and Slovenia respectively). The costs for operations and labour were lower for Italy (–€41 ha^{-1}) as the labour costs ha^{-1} for *Trichogramma* release (+€6 ha^{-1}) were much lower than the operation costs for the insecticide application (+€46 ha^{-1}), whereas in France and Slovenia the costs for operations and labour were only €4 ha^{-1} and €2 ha^{-1} higher (labour for *Trichogramma* release) than the untreated CON (Fig. 7A). However, statistical analysis of the total costs per country showed no significant difference between years, ECB management regimes or their interaction. With regard to the gross margin, in Italy, only a significant year difference was determined, with no difference between TRI and insecticide treatment (Fig. 7B). In France, no significant differences in gross margin were found between TRI and untreated. In contrast, in Slovenia, ANOVA determined significant difference in gross margin between years ($P < 0.001$), TRI versus untreated ($P < 0.01$) and their interaction ($P < 0.05$). The average gross margin (i.e. mean of 2 years) of biological control with *T. brassicae* was significantly lower than the CON untreated management in Slovenia (–€150 ha^{-1}), and higher in Italy (+€7 ha^{-1}) and France (+€84 ha^{-1}), although, as mentioned before, the latter two differences were not significant.

The SYNOPS calculations showed that, in a situation with small buffer zones (1 m) and drift reduction of 50%, as in the case of Italy, insecticide spraying would cause a high risk for aquatic life (Table 3). The risks could be reduced by increasing the width of the buffer zone and by reducing the drift (e.g. by using low-drift nozzles and air support systems).

4 DISCUSSION

This study, conducted in real field conditions on commercial or demonstration farms in different geographic and climatic regions, demonstrates the diversity of ECB pressure across Europe and partly explains the choice of insecticide applications as the conventional management against this pest in Italy. Results from ECB adult catches by light traps, maize damage from G1 and the number of G2 egg masses showed that northern Italy suffers the highest ECB pressure among participating countries, followed by southern France and Slovenia (Fig. 2 and Table 2).

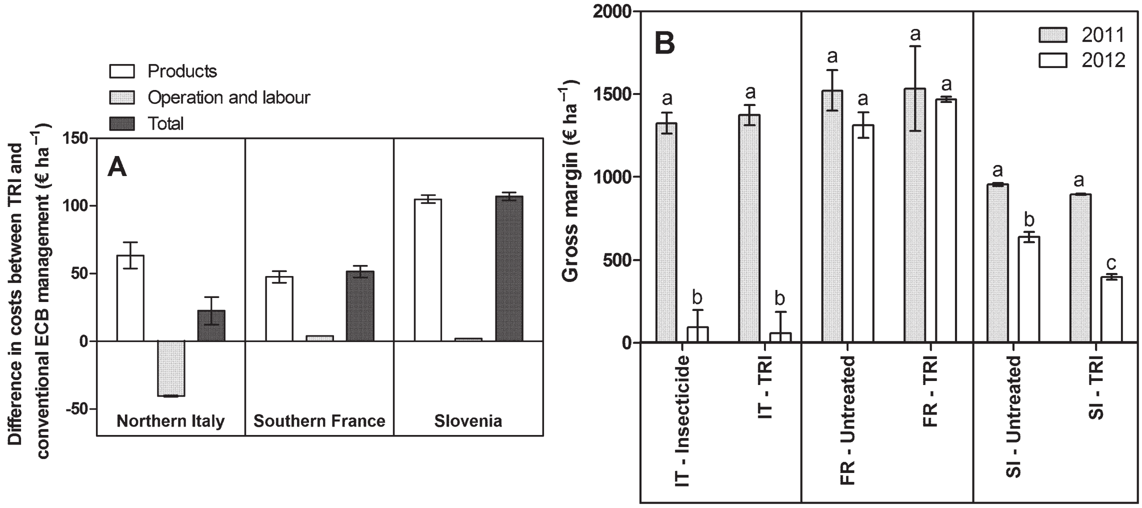

Figure 7. The difference in costs between biological control with *T. brassicae* (TRI) and conventional ECB management (insecticide treated and untreated) (A), and the gross margin (B, absolute value) of TRI and CON treatment in individual countries. Presented data are means ± standard error from all on-farm experiments per country during the 2 years of the study (A), and means of individual years per ECB management and country (B). ANOVA was performed on a country level. Mean values per country not sharing the same lower-case letter are significantly different at $P < 0.05$ according to Fisher's protected LSD test. IT, northern Italy; FR, southern France; SI, Slovenia.

Table 3. SYNOPS exposure toxicity ratio values[a] (aquatic and terrestrial life) when spraying the insecticide lambda-cyhalothrin and as affected by the width of the buffer zone and drift reduction by the spraying technique. Data presented are average values of farms and years in Italy ± standard error

	Acute risk		Chronic risk	
Application conditions	Aquatic life	Terrestrial life	Aquatic life	Terrestrial life
Buffer zone = 1 m, drift reduction = 50%	0.50 ± 0	0	41.40 ± 0.06	0
Buffer zone = 5 m, drift reduction = 90%	0.03 ± 0	0	2.25 ± 0.004	0

[a] For low environmental risk, values should be <0.1 for acute risk and <1.0 for chronic risk.

Predicting maize plant damage just by studying the abundance of adults is unreliable.[32] The main importance of the first generation is the high reproductive rate of the individuals, because one corn borer female can lay up to 300 eggs.[32] The second generation is thus more numerous, and as such has more direct consequences on maize plant damage. It is only by assessing the number of G2 egg masses that the time of *T. brassicae* release can be optimised. Ideally, the timing of egg parasitoid release should anticipate peak ECB oviposition.[23] Asynchrony between parasitoid release and ECB oviposition activity can reduce parasitism efficiency.[19,23] The method of determining the time of release of *T. brassicae* in this study was satisfactory. Results show that the release dates in the various countries coincided well with the beginning of the more damaging G2 in all countries, as the parasitism rate of *T. brassicae* was above 63% in most experiments, even reaching 83% in Italy in 2011 (Fig. 3), and was comparable with previously reported studies.[24] The extremely high temperatures in Italy and Slovenia in 2012 principally affected Slovenia (only 17% parasitism). Previous studies have demonstrated the negative effects of high temperatures on efficacy of *T. brassicae*[33,34] and *T. ostriniae*.[23,35] However, it should be taken into account that the use of insecticides as the conventional management also does not guarantee 100% ECB control in the respective countries because there is a limited 'window' for application, and farmers usually depend on the availability of contractors to apply insecticides with high-clearance ground sprayers. Besides, ECB is difficult to control because larvae are only exposed to insecticide sprays from egg hatching until larval tunnelling into maize plants or ears.[24] Insecticide applications thus have an even shorter timeframe for efficient ECB control than *Trichogramma* spp. release. Systemic insecticides could potentially exhibit a greater efficacy against ECB because of a longer residual time in plant tissues, but they could also detrimentally affect the natural enemies of this pest.[2,24,36] In this respect, the use of *Trichogramma* spp. against ECB does not directly damage autochthonous populations of generalist predators and other beneficial arthropods,[24,36] but also alleviates the negative short- and long-term effects of insecticide use on farmers' health and the environment.[24] Even if risk reduction measures (e.g. wider buffer zones, 90% drift reduction) were to be implemented in Italy, the chronic risks of lambda-cyhalothrin use to aquatic life would still be too high (Table 3).[31] Therefore, with regard to environmental risks biological ECB control is a better solution.

Results obtained for ECB damage on final maize stand and ears did not show a higher efficacy of *T. brassicae* release compared with insecticide treatment or no treatment. The same non-significant effect between ECB management regimes was observed on grain yields, stressing that biological control of ECB could result in yields similar to conventional management in the respective countries. Several studies worldwide have reported significantly higher grain yield increases when *Trichogramma* spp. were released against corn borers.[24,26]

Biological control with *T. brassicae* maintained mycotoxin levels in grain below the EU threshold for maize raw materials destined for food products (4000 μg kg^{-1} grain for fumonisin B1 and B2 in raw maize).[37] No significant differences were observed in mycotoxin concentrations between TRI and CON plots. This is of importance, especially for Italy where overall higher mycotoxin levels were observed compared with the other countries, in spite of insecticide application being the conventional management strategy. The only exception was one farm in Italy in 2012, where more than 11 000 μg fumonisins kg^{-1} grain was measured in both CON and TRI plots. This isolated case was most probably caused by extremely high temperatures and lack of irrigation. Investigating the interaction between ECB damage on ears, *Fusarium* spp. presence on ears and mycotoxin concentration showed that all parameters were positively correlated with one another, which agrees with previous research demonstrating that ECB can vector fungal infection (i.e. *F. verticillioides*) through kernel wounds and spread the infection during larval movement,[37,38] thus proportionally influencing the mycotoxin contamination in grain. It can therefore be assumed that the parameter ECB damage to ears can be a useful indicator for potential mycotoxin contamination in grain.

The economic impact of biological control compared with conventional management (insecticide treated or untreated) is determined by changes in costs and crop yields. For all trials, the total variable costs of biological control with *T. brassicae* were higher than those for conventional management, mainly owing to the cost of the *Trichogramma* product. However, effects were not found to be significant. The average gross margin associated with biological control was not significantly affected for France, where only one release was performed in TRI plots and where CON plots were untreated, and in Italy, where two releases were performed in TRI plots, and where an insecticide was applied in the CON plots. In contrast, *Trichogramma* release was found to be significantly not economically sustainable in Slovenia owing to the high cost of *Trichogramma* product, as two releases were performed and lower yields obtained in 2012 (Fig. 7). Accordingly, in situations where an insecticide application is necessary for ECB control, the biological control by *Trichogramma* release seems to be a suitable alternative.

5 CONCLUSIONS AND PERSPECTIVES

Trichogramma brassicae inundative biological control seems to be a rational choice when ECB pressure exceeds economic thresholds in countries where insecticides are generally not used to combat this pest. In southern France, biological control with one optimally timed *T. brassicae* release improved ECB control slightly compared with the untreated conventional management, without reducing the gross margin. In Slovenia, however, because of the two releases performed, the TRI strategy was not economically sound. More importantly, in northern Italy, even though biological ECB control was slightly inferior to lambda-cyhalothrin application, this did not cause any yield or economic losses, maintained mycotoxin levels below the EU threshold for maize raw materials destined for food products and also alleviated the negative effects of insecticide use on farmers and the environment.[24]

For Italy and Slovenia, as well as other maize-producing countries considering *Trichogramma* deployment, further research investigating the efficacy of one *T. brassicae* release is needed to reduce the costs of application and make this tool even more attractive to farmers. To do this, the development and use of effective forecasting and decision support systems are needed to optimise application, keep to one *T. brassicae* release and consequently reduce the costs involved. This is in keeping with IPM principle No. 3 stated in Annex III of Directive 2009/128/EC.[13]

Generally, policy- and decision-makers should provide support by more closely involving the regional advisory services for the successful dissemination and implementation of biological control (e.g. organise open field visits, establish farmer training programmes and encourage the formation of farmer groups to exchange experiences of IPM implementation), but also through possible subsidy schemes to motivate farmers to adopt this IPM tool and compensate for possible reductions in their gross margins owing to the higher costs of the *Trichogramma* product.[39] A good example comes from south-western Germany (federal state of Baden Württemberg), where the use of biological control with *T. brassicae* against ECB in maize is currently subsidised with €60 ha^{-1} (Verschwele A, private communication, 2015).

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