Efficacy of Bt Maize against European Corn Borer in Central Europe

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Abstract

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The efficacy of Bt maize MON 810-YieldGard® and of *Trichogramma* wasp against European corn borer (ECB) (*Ostrinia nubilalis* Hübner) was evaluated in the period of 2002–2008 in field trials on three localities in the Czech Republic. The efficacy of Bt maize on the reduction of the number of tunnels caused by ECB per 100 maize plants before harvest was always 100% and that in *Trichogramma* treatment was on average 50%. The mean increase of the yield of 15% and 10% was obtained in Bt maize and *Trichogramma* treatments, respectively in comparison with the untreated control. The damage curve and economic injury level by ECB on maize was developed for the evaluation of the yield losses and management of the pest control. The higher economic efficacy of growing Bt maize as compared to other control measures is documented.

Keywords: MON 810; *Ostrinia nubilalis*; transgenic maize; *Trichogramma*; economic injury level

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a major pest of maize (*Zea mays* L.) developing one generation per year in Central Europe, while in the Mediterranean region, ECB produces 2–3 generations per year (Velasco *et al*. 2007). ECB larvae cause damage to the stalks and ears, which results in yield losses and reduced quality of the grain production. The plants damaged by ECB are susceptible to secondary infections by *Fusarium* spp. and other pathogens producing mycotoxins (Munkvold & Desjardins 1997). In the areas highly infested with ECB, the yield losses in Europe without control measures range usually between 5% and 30% (Meissle *et al*. 2010). The population density of ECB and the injury occurrence caused by ECB has been increasiog in the Czech Republic since 2000 (Anonymous 2012).The yield losses caused by ECB in the Czech Republic are estimated to range from 10% to 20% at least on half the area of grain maize. In 2008, 100 000 ha was sown with grain maize in the Czech Republic and more than 50% of this area was treated with insecticides against ECB. The options for insecticides reduction are the biological control of ECB, mainly by *Trichogramma* spp. wasps, and the use be genetically modified maize – Bt maize (Meissle *et al*. 2010). Bt maize is one of the tools in the integrated pest management (IPM). Bt maize is a highly specific and highly efficient pest control measure that allows the growers to produce high-quality grain with reduced insecticide application and farm operations (Meissle *et al*. 2011). Bt maize hybrid MON 810 is genetically engineered to express *Cry1Ab* gene that naturally occurs in the soil bacterium *Bacillus thuringiensis* (Bt). The production of this toxin provides the protection of the maize plants against ECB larvae (e.g. Burkness *et al*. 2002). Gene promoters regulate the tissue-specific expression of the *Bt* gene. MON 810 uses a gene promoter, which results in a season-long expression of the Bt toxin in all plant tissues (Archer *et al*. 2000). In the European Union, Bt maize hybrids were planted in 2010 on about 90 000 ha in Spain, Portugal, the

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Czech Republic, Poland, Slovakia, and Romania (James 2010). In the Czech Republic, where Bt maize hybrid MON 810 has been permitted since 2005, the use of Bt maize increased from 270 ha to 8380 ha in 2008. In 2009 and 2010, the Bt maize area stagnated at ca. 5000 ha (Anonymous 2011) due to the problems with Bt maize export to EU countries.

Many studies have demonstrated high efficacy of Bt maize against ECB (Koziel *et al.* 1993; Pilcher *et al.* 1997; Burkness *et al.* 2002). The yield-losses relations and economic injury level for ECB were established in the field studies by Bode and Calvin (1990) in accordance with the definition of the economic injury level published by Pedigo *et al.* (1986). The efficacy and risk efficiency of sweet corn hybrids expressing a *Bacillus thuringiensis* toxin for Lepidoptera pest management were established by Burkness *et al.* (2002). The economics of Bt maize, risk and the value of Bt maize were evaluated by Hyde *et al.* (1999), Mitchell *et al.* (2002) and Hurley *et al.* (2004). The cumulative benefits of BT maize over 14 years of growing in the USA for maize growers were established by Hutchison *et al.* (2010). Bt maize growing on 63% of the area in the USA in 2009 suppressed ECB also on the areas of non-Bt maize. Communal benefits of transgenic maize as a reasult of the so called "halo effect" were explained by Tabashnik (2010). Based on the conditions in Europe, Magg *et al.* (2001) published a comparison of Bt maize hybrids with their non-transgenic isolines and commercial varieties in view of the resistance to ECB and agronomic traits.

In this study, we evaluated the biological efficacy of Bt maize and the biological control using *Trichogramma* as an alternative to synthetic pesticides for the ECB control. The field trials were carried out from 2002 to 2008 at three localities. The main aim of the study was to establish the economic injury level for ECB in the conditions of Central Europe, to enable the prediction of the losses caused by ECB and the prediction of the expected benefits due to the Bt maize growing. The specific aims of the study were to (1) evaluate the biological efficacy of Bt maize hybrid MON 810 with the untreated non-Bt isoline and non-Bt isoline treated with *Trichogramma* wasp, (2) determine the agronomical characteristics (the maize yield), (3) determine the damage curve as the dependence between the level of the plant injury or percentage of the injured plants and the percentage of the yield reduction, (4) determine the economic injury level, and (5) evaluate the abundance of ECB before the adoption of Bt maize.

MATERIAL AND METHODS

Field trials. The field trials were conducted in the period of 2002–2008 in three localities in the Czech Republic: Prague (50°4'58''N, 14°18'33''E, 364 m a.s.l.) in 2002–2004, Čáslav (49°54'40''N, 15°25'7''E, 231 m a.l.s.) in 2005–2008, and Ivanovice na Hané (49°18'41''N, 17°5'30''E, 214 m a.s.l.) in 2002–2008. The experimental field near Prague is located in Central Bohemia, Čáslav is located in East Bohemia, and Ivanovice na Hané is located in South Moravia. Three variants of ECB control were tested: (1) Bt maize hybrid MON 810 DKC3421YG (producing Cry1Ab toxin from *Bacillus thuringiensis*) (Bt maize treatment), (2) non-Bt isoline maize hybrid Monumental DKC3420 treated with *Trichogramma* (*Trichogramma* treatment), (3) non-Bt isoline maize hybrid Monumental DKC3420 without treatment (untreated control). Each variant was tested on a 0.25 ha plot. *Trichogramma* wasps were released in the preparation Trichocap in the amount of 3 × 100 capsules per ha. The first application of Trichocap was timed according to the monitoring of the first flight activity of the moth by light traps; the second application was made 10 days later.

Damage and response variables. Fifty to seventy plants were evaluated in each variant. The following traits were determined: (1) stalk breakage below the ear (BBE), (2) stalk breakage above the ear (BAE), (3) number of tunnels in the ear (TIE), (4) number of tunnels in the whole plant (TT), (5) incidence of *Fusarium* spp. in the ear by visible symptoms, (6) biological efficacy of the ECB control used, (7) grain yield in t/ha. The plant injury caused by ECB larvae was measured by longitudinal splitting of the stalks and checking of the ears before harvest.

Determination of economic injury level. The damage curve describes the relationship between the yield and injury. The economic injury level (EIL) was defined as the lowest population density that will cause economic damage (Pedigo *et al.* 1986). We used a modified theoretical model of EIL according to Pedigo *et al.* (1986), defined as $EIL = C/(V \times D)$, where: EIL – number of injury equivalents, i.e. the number of tunnels per

100 plants before harvest, that corresponds to the number of pests per 100 plants before harvest in the untreated hybrid, *C* – cost of the management activity per unit of producion (EUR/ha) and includes premium costs for Bt maize seeds (technology fee) and the costs from the biological control by *Trichogramma* wasp, V – market value (utility) per unit of production (EUR/ha), and D – damage function measured as the summarised yield loss per unit of pest (ECB). The calculation of V is V = Y × P, where Y – yield (t/ha), P – price of grain maize (EUR/t). We used as Y extreme values of yield of Bt maize from the trials and extreme values of average yield of grain maize in 2000–2010 period in the Czech Republic. We used for P the average and range of purchase price of grain in the Czech Republic during 2000–2010. The damage curve was calculated from data originated from our field trials. The linear regression was used to construct the damage curve using the equation $y = A_0 + A_1 \times x$ as the relation between the proportion of the yield reduction (y) and the number of ECB per 100 plants (x). EIL = ((100 × C × e) + (A_0 × V))/(A_1 × V), where e = 100/percentage of efficacy. For the efficacy of 100% (Bt maize) e = 1, for the efficacy of 50% (*Trichogramma*) e = 2, and for the efficacy of 85% (selective insecticides) e = 1.176. The economic and agronomic parameters of three control stategies against ECB in maize, i.e. Bt maize, *Trichogramma* wasp, and selective insecticides, are as follows: C = the cost of the management activity per unit of production (EUR/ha), C_1 = 35 EUR (premium cost for Bt seeds), C_2 = 50 EUR (the cost of biological control by *Trichogramma* wasp), and C_3 = 40 EUR (the cost of selective insecticides); V = Y × P (market value (utility) per unit of production (EUR/ha)), where V_1 to V_{12} are combinations of different grain yields (Y in t/ha) and different grain prices (P in EUR/t). Y_1 = 5.6 t/ha is the lowest yield of grain in Bt maize field trials in this study (2002–2008), Y_2 = 7 t/ha is the lowest average yield of grain maize in the Czech Republic (from 2002 to 2011), Y_3 = 10.2 t/ha is the highest average yield of grain maize in the Czech Republic (from 2002 to 2011), Y_4 = 13.2 t/ha is the highest yield of Bt maize grain in the field trials in this study (2002–2008). P_1 = 100 EUR/t is the lowest average price of grain maize in the Czech Republic (2000–2011), P_2 = 150 EUR/t is the average price of maize grain in the Czech Republic (2002–20011), and P_3 = 200 EUR/t is the highest average price of maize grain in the Czech Republic (2002–2011).

Evaluation of abundance of ECB and regression model for growth rate. We measured larval abundance per 100 plants in non-Bt maize before harvest in the field trials in Ivanovice na Hané (2002–2008), Prague (2002–2004), and Čáslav (2005–2008). In these trials, Bt maize was grown on very small areas, essentially as islands within a non-Bt maize landscape. Hence, we can consider this period as the period before Bt maize adoption in the Czech Republic. Larval mortality increases with larval density, and the population growth more generally depends inversely on density. We estimated the annual per capita growth rate, similarly as Hutchison *et al.* (2010). The linear regression was used for the construction of the model as the relation between the number of ECB larvae per 100 plants and per capita growth rate (r), where r = ln (Nt/Nt – 1).

Data analysis. The XL-STAT 2009 program (Addinsoft USA, New York, USA) and one-way ANOVA analysis were used to evaluate the effect of the maize treatment on the stalk breakage and number of tunnels in maize caused by ECB, and the incidence of *Fusarium* spp. in the ears. The biological efficacy of the maize treatment on the reduction of the number of tunnels in maize plant caused by ECB in comparison with the untreated control was calculated by the formula $x_1 = 100 - [(100 \times y_1)/z_1]$, where: x_1 – biological efficacy, y_1 – number of tunnels/100 plants in the treated variant (Bt maize, *Trichogramma*), z_1 – number of tunnels/100 plants in the untreated control. The linear regression was used also to describe the relation between the number of tunnels per 100 plants and the percentage of injured plants before harvest. Multi-factor ANOVA was used to test the differences in the yield of grain between variants and years in particular localities. The data from Ivanovice na Hané in 2002 and Čáslav in 2007 were excluded from this analysis because of the missing data from *Trichogramma* treatment.

RESULTS

Plant injury caused by ECB

In our trials, no injury by ECB, expressed as the numbers of broken stalks and tunnels in the plants and ears, was observed in Bt maize hybrid in all the years and localities (Table 1). The *Trichogramma* treatment resulted in significantly ($P < 0.05$) lower numbers of broken stalks below the ear than found with the untreated non-Bt hybrid, and except in the Čáslav locality, it showed also lower numbers of stalks broken above the ear (Table 1). The mean number of stalks broken below the ear ranged from 0 to 3.0 (on average 1.3) per 100 plants for the *Trichogramma* treatment, but the ranged from 1.0 to 7.0 (on average 4.0) per 100 plants in untreated, non-Bt maize. The number of stalks broken above the ear ranged from 0.3 to 8.0 (on average 5.4) per 100 plants in *Trichogramma* treatment, while it ranged from 3.0 to 15.0 (on average 8.3) per 100 plants in the untreated Bt maize. The numbers of tunnels caused by ECB in the ears and in the whole plants in the *Trichogramma* treatment were significantly (P < 0.05) lower than in the untreated ones in all localities except Prague, where the number of tunnels in the ears did not differ significantly between the untreated control and *Trichogramma*-treated plants (Table 1). In the untreated control, the average number of tunnels in the whole plants was 70, 102, and 149 per 100 plants in Čáslav, Prague, and Ivanovice na Hané, respectively. In *Trichogramma*-treatment, the mean number of tunnels decreased by approximately 50% in all localities (Table 1).

Incidence of *Fusarium*

In our trials, significantly (P < 0.05) lower number of ears with *Fusarium* spp. occurrence was found in Bt maize treatment in comparison to *Trichogramma*-treated and untreated plants (Table 1). The incidence of *Fusarium* spp. in the ears differed according to the locality, the lowest incidence on Bt maize having been observed in the locality of Prague (Table 1). The incidence of *Fusarium* spp. in the ears was reduced in Bt maize by 100, 53, and 40% in Prague, Čáslav, and Ivanovice na Hané, respectively. By contrast, the incidence of *Fusarium* spp. in *Trichogramma*-treated and untreated maize was not significant in any locality. For *Trichogramma* treatment, the incidence of *Fusarium* spp. in the ears was reduced only by 8, 0, and 8% in Prague, Čáslav, and Ivanovice na Hané, respectively.

Abundance of ECB and biological efficacy of Bt maize and Trichogramma wasp

No ECB larvae were found before harvest in Bt maize in the field trials during 2002 and 2008. The

Table 1. Means (± SEM) for ECB control provided by Bt maize or *Trichogramma* spp., evaluated for five response variables in Prague, Ivanovice na Hané, and Čáslav

Locality	Variant	BBE	BAE	TIE	TT	F
Prague	Bt maize	0^a	0^a	0^a	0^a	0^a
	non-Bt *Trichogramma*	$3.0\ (1.2)^b$	$0.3\ (0.1)^b$	$11.0\ (2.2)^b$	$53.0\ (5.9)^b$	$12\ (6.0)^b$
	non-Bt untreated	$7.0\ (1.8)^c$	$3.0\ (1.2)^c$	$11.0\ (2.2)^b$	$102.0\ (8.8)^c$	$13\ (6.3)^b$
	ANOVA model	F = 361.27 df = 2 R^2 = 0.574	F = 255.08 df = 2 R^2 = 0.488	F = 733.14 df = 2 R^2 = 0.742	F = 806.36 df = 2 R^2 = 0.750	F = 351.44 df = 2 R^2 = 0.567
Ivanovice na Hané	Bt maize	0^a	0^a	0^a	0^a	$53\ (10.5)^a$
	non-Bt *Trichogramma*	$1.0\ (0.6)^b$	$8.0\ (1.6)^b$	$16.0\ (2.2)^b$	$71.0\ (7.7)^b$	$113\ (21.7)^b$
	non-Bt untreated	$4.0\ (1.1)^c$	$15.0\ (2.0)^c$	$25.0\ (2.5)^c$	$149.0\ (9.1)^c$	$112\ (19.5)^b$
	ANOVA model	F = 455.45 df = 2 R^2 = 0.426	F = 1095.59 df = 2 R^2 = 0.641	F = 1455.04 df = 2 R^2 = 0.703	F = 385.20 df = 2 R^2 = 0.386	F = 85.97 df = 2 R^2 = 0.123
Čáslav	Bt maize	0^a	0^a	0^a	0^a	$73\ (11.3)^a$
	non-Bt *Trichogramma*	0^a	$8.0\ (1.9)^b$	$7.0\ (1.8)^b$	$45.0\ (6.3)^b$	$112\ (16.6)^b$
	non-Bt untreated	$1.0\ (0.7)^b$	$7.0\ (1.6)^b$	$12.0\ (2.0)^c$	$70.0\ (5.7)^c$	$122\ (20.4)^b$
	ANOVA model	F = 65.31 df = 2 R^2 = 0.172	F = 111.72 df = 2 R^2 = 0.263	F = 107.32 df = 2 R^2 = 0.255	F = 134.27 df = 2 R^2 = 0.300	F = 23.56 df = 2 R^2 = 0.070

BBE – number of stalks broken below the ear; BAE – number of stalks broken above the ear; TIE – number of tunnels in the ear; TT – number of tunnels in the whole plant; F – number of ears with *Fusarium* spp. occurrence; all calculations are made per 100 plants; values with different letters in columns denote statistically significant difference at P < 0.05

Table 2. Larval abundance of ECB per 100 plants in untreated (C) and in *Trichogramma* treatment (T) after application of *Trichogramma* wasp and biological efficacy of treatment with *Trichogramma* wasp (E) in the field trials at Ivanovice na Hané (2002–2008), Prague (2002–2004), and Čáslav (2005–2008)

Locality	Year	C	T	E
Prague	2002	95	48	49.47
	2003	57	43	24.56
	2004	155	68	56.13
	mean	**102**	**53**	**43.00**
Ivanovice na Hané	2002	253	108	57.31
	2003	130	88	32.31
	2004	155	68	54.67
	2005	132	107	18.94
	2006	101	17	83.17
	2007	74	40	45.95
	2008	195	66	66.15
	mean	**149**	**71**	**51.00**
Čáslav	2005	140	142	0.00
	2006	31	6	80.65
	2007	50	x	x
	2008	60	18	53.33
	mean	**70**	**59**	**45.00**

x – missing data from *Trichogramma* treatment

biological efficacy of Bt maize, as indicated by the reduction in the tunnels number caused by ECB (per 100 maize) plants was always 100%. The biological efficacy of *Trichogramma* wasp on the reduction of the number of tunnels per 100 plants in comparison to Bt maize largely varied between the localities and years and ranged from 19% in Ivanovice na Hané in 2005 to 83% in Ivanovice na Hané in 2006 (Table 2).

Yield of grain

The mean yields of grain in particular localities differed significantly between years and variants (Table 3). In general, the highest yields of grain were obtained in the Ivanovice na Hané locality. The grain yields were higher in most cases with Bt maize and the *Trichogramma* treatments than in the untreated control. In all three localities, the yields of Bt maize were the highest in all years. The mean increases in the yield, of 18, 12, and 11% in Bt maize treatment, and 9, 8, and 13% in *Trichogramma* treatment in comparison to the untreated control were obtained in Prague, Čáslav, and Ivanovice na Hané localities, respectively. However, the increase in yield largely varied between the years. In the Čáslav locality, the yield was increased by 30% in Bt maize treatment, while in the Prague locality in 2003, the yield was even decreased for 4.5% in *Trichogramma* treatment in comparison to the untreated control. Hence, the yield increase in Bt maize and *Trichogramma* treatments highly depended on the conditions of the respective year.

In general, the biological efficacy of Bt maize on the reduction of tunnels number per plant was

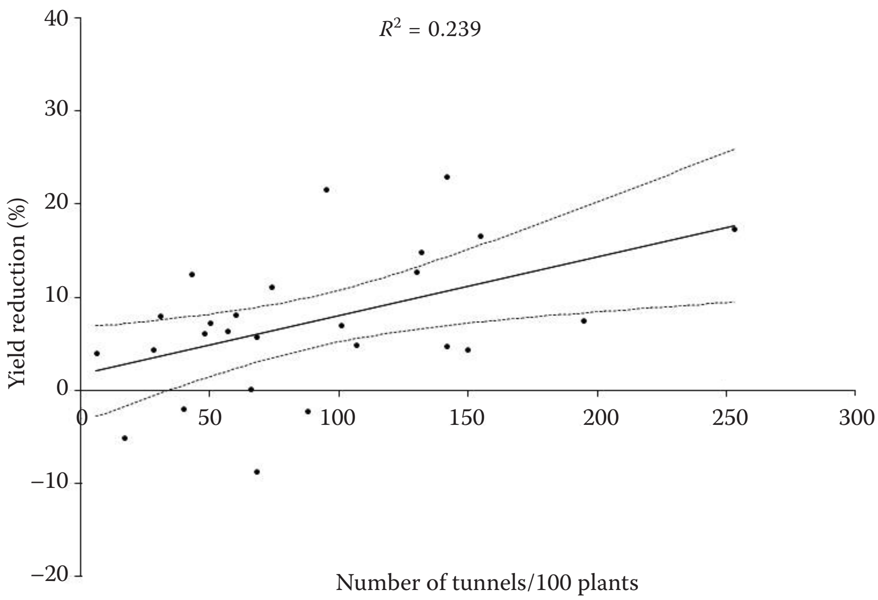

Figure 1. Linear regression of number of tunnels per 100 plants and percentage of yield reduction – data from Čáslav (2005–2008) and Ivanovice na Hané (2005–2008)

Table 3. Means (SEM) for yield of grain (t/ha) on Bt maize, non-Bt maize with *Trichogramma* and untreated maize in Prague, Ivanovice na Hané, and Čáslav in 2002–2008

Locality	Year	Bt maize	*Trichogramma*	Untreated plants
Prague	2002[c]	11.5 (0.4)	10.8 (0.3)	9.0 (0.3)
	2003[a]	7.6 (0.3)	6.7 (0.2)	7.1 (0.2)
	2004[b]	8.3 (0.3)	7.8 (0.4)	6.9 (0.4)
	mean	**9.1*****	**8.4****	**7.7***
ANOVA model		F = 143.04, df = 8, R^2 = 0.97		
Ivanovice na Hané	2003[b]	9.0 (0.3)	9.2 (0.4)	7.9 (0.3)
	2004[a]	7.3 (0.3)	7.9 (0.3)	6.9 (0.5)
	2005[c]	11.4 (0.6)	10.8 (0.8)	9.7 (1.4)
	2006[e]	13.2 (0.5)	13.8 (1.0)	12.2 (1.0)
	2007[d]	12.6 (0.5)	12.9 (0.5)	11.2 (0.4)
	2008[d]	12.0 (0.4)	11.9 (0.5)	11.1 (0.7)
	mean	**10.9****	**11.1****	**9.8***
ANOVA model		F =130.84, df = 7, R^2 = 0.92		
Čáslav	2005[a]	5.6 (0.5)	5.3 (0.5)	4.3 (0.3)
	2006[c]	12.4 (0.5)	11.9 (0.1)	11.4 (0.6)
	2008[b]	11.8 (0.2)	11.3 (0.1)	10.9 (0.2)
	mean	**9.9*****	**9.5****	**8.8***
ANOVA model		F = 662.43, df = 4, R^2 = 0.99		

Values with different letters in columns denote statistically significant difference between years (P < 0.05); values with different number of asterisk in rows denote statistically significant difference between variants (P < 0.05)

always 100% which brought an increase in the yield bt 16% on average. The biological efficacy of *Trichogramma* wasp on the reduction of tunnels number per 100 plants was 48% which brought an increase of the yield by 10% on average.

Damage curve

The damage curve is calculated as a linear regression model according to the equation $y = 1.653 + 0.063 \times x$ expressing the relation between the percentage of the yield reduction (y) and the number of tunnels per 100 plants (x) (F = 7.53, df = 24, R^2 = 0.24), indicating 5% yield reduction at 53.13 tunnels per 100 plants (Figure 1). Nonlinear regression model following the equation $y = 17.407 \times \mathrm{Exp}(0.026x)$ expressed the relation between the number of tunnels caused by ECB per 100 plants (y) and the percentage of injured plants (x) (df = 116, R^2 = 0.86). According to this model, the number of tunnels per 100 plants increased with the percentage of injured plants. Hence, 44 tunnels per 100 plants indicate 50% of injured plants (Figure 2).

Economic injury level

The values of EIL varied most with the grain price, less with the efficacy of the control measures, and the least with the grain yield (Table 4). With the knowledge of the average plant injury before harvest in a given locality or region, it is possible to plan the management of the pest control for the next year to achieve high economic efficacy. According to Figure 2 and the corresponding equation, it is possible to estimate EIL only on the basis of known plant injury, this being a simple method for practical application. At a high price of grain and average yields, the EIL is 53, 63, and 104 for Bt maize, selective insecticides and *Trichogramma* wasp, respectively. It corresponds to 42, 48, and 60% of injured plants before harvest. At a lower incidence of ECB, the control measures used are not economically effective for the parameters given in Table 4. With the decrease of grain price the EIL values increase and the economic efficacy of the control measures used decrease. At average price of grain and average yields EIL is 79, 97, and 177 for Bt maize, selective insecticides, and

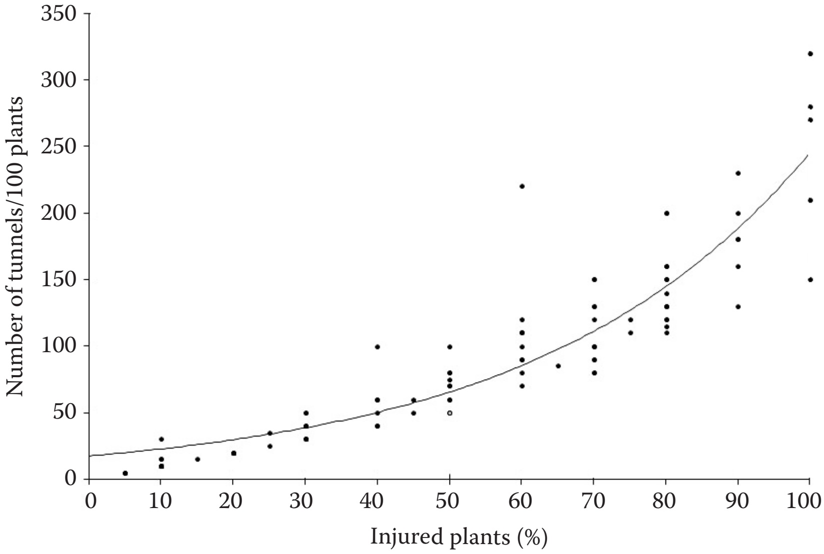

Figure 2. Nonlinear regression of percentage of injured plants and number of tunnels per 100 plants

Trichogramma wasp, respectively. It corresponds to 57, 65, and 88% of injured plants before harvest. At incidence of 60% and a greater proportion of the injured plants before harvest, the Bt maize growing or selective insecticides against application ECB are economically effective and, with the increase of plant injury, the economic efficacy of the control measures used increase.

Abundance of ECB in non-Bt maize and regression model for growth rate

The larval abundances of ECB per 100 plants in non-Bt maize before harvest in the field trials in Ivanovice na Hané (2002–2008), Prague (2002–2004), and Čáslav (2005–2008) are given in Table 2. In Ivanovice na Hané, the average abundance was 149,

Table 4. Values of EIL and plant injury (in %) for economic and agronomic parameters of field trials and for average and extreme values of economic parameters in the EU for control of ECB with Bt maize and *Trichogramma* wasp and selective insecticides

Input in model P (EUR/t)	Input in model Y (t/ha)	Input in model market value V = Y × P (EUR/ha)	Bt maize – efficacy 100% cost C_1 = 35 EUR		*Trichogramma* wasp – efficacy 50% cost C_2 = 50 EUR		Selective insectides – efficacy 85% cost C_3 = 40 EUR	
			EIL	injured plants (%)	EIL	injured plants (%)	EIL	injured plants (%)
P_1 = 100	Y_1 = 5.6	V_1 = 560	125	75	310	100	160	84
P_1 = 100	Y_2 = 7	V_2 = 700	106	68	253	100	133	77
P_1 = 100	Y_3 = 10.2	V_3 = 1020	81	58	182	88	99	66
P_1 = 100	Y_4 = 13.2	V_4 = 1320	68	52	147	80	83	59
P_2 = 150	Y_1 = 5.6	V_5 = 840	92	63	215	95	115	71
P_2 = 150	Y_2 = 7	V_6 = 1050	79	57	177	88	97	65
P_2 =150	Y_3 = 10.2	V_7 = 1530	62	48	130	76	75	55
P_2 =150	Y_4 = 13.2	V_8 = 1980	54	43	106	68	64	49
P_3 = 200	Y_1 = 5.6	V_9 = 1120	76	55	168	85	93	63
P_3 = 200	Y_2 = 7	V_{10} = 1400	66	50	140	79	80	57
P_3 = 200	Y_3 = 10.2	V_{11} = 2040	53	42	104	67	63	48
P_3 = 200	Y_4 = 13.2	V_{12} = 2640	47	38	86	60	55	43

EIL – number of ECB per 100 plants before harvest/percentage of plants injured by ECB before harvest in Prague 102, and in Čáslav 70 larvae per 100 plants, respectively. The linear regression model using the equation $r = 3.502 - 0.781 \times \text{Nt}$ ($F = 6.07$, df = 9, $R^2 = 0.401$) expressed the relation between per capita growth rate (r), where $r = \ln(\text{Nt}/\text{Nt} - 1)$, and the number of ECB larvae per 100 plants (Nt) before the adoption of Bt maize in Central Europe (Figure 3).

DISCUSSION

Plant injury caused by ECB and biological efficacy of Bt maize and Trichogramma wasp

Several studies conducted in the USA and Europe reported the high level of resistance of Bt maize against ECB (Koziel *et al.* 1993; Archer *et al.* 2000; Magg *et al.* 2001). Magg *et al.* (2001) found a significantly lower number of broken stalks and a lower number of ECB larvae per plant in Bt maize hybrids as compared to non-Bt hybrids in Germany. However, the biological efficacy of Bt maize on the reduction of the number of tunnels caused by ECB was below 100%. In our experiments, the biological efficacy of Bt maize on the reduction of the number of tunnels caused by ECB was always 100%. After the application of *Trichogramma* wasp, total injury of plants decreased in comparison to the untreated control by 48, 48, and 52% in Čáslav, Prague, and Ivanovice na Hané, respectively.

Bt maize also showed a reduced contamination with *Fusarium* sp. compared with non-transgenic hybrids (Munkvold *et al.* 1999). The quality of grain and incidence of micromycets and mycotoxins are highly influenced by the injury of ears caused by ECB. The average injury of ears caused by ECB in the untreated control was, in our trials, 11, 12, and 25% in Prague, Čáslav, and Ivanovice na Hané, respectively. The ears of Bt maize were not injured by ECB in any locality. After the application of *Trichogramma* wasp, the injury of ears in comparison with the untreated control did not decrease in the locality of Prague with it decreased by 42 and 36% in the localities Čáslav and Ivanovice na Hané, respectively. The average biological efficacy of *Trichogramma* wasp on reduction of plant injury caused by ECB was 48%. The biological efficacy of *Trichogramma* wasp varied more between particular years than between localities. Despite the relatively low efficacy in comparison to Bt maize, *Trichogramma* wasp application significantly increased the yield of grain in all localities (Table 2). We recorded a comparable increase of yield in similar field trials after the application of selective insecticides Steward (a.i. indoxacarb) and Integro (a.i. methoxyfenozide) with average biological efficacy of 85% (unpublished data). We used this value of the biological efficacy of selective insecticides for the determination of EIL (Table 4). The biological control of ECB with *Trichogramma* wasp is one alternative to reduce insecticidal application. According to Meissle *et al.* (2010), the efficacy higher than 75% destroyed pest eggs, and the price 35–40 EUR per hectare ise comparable to that of insecticides unless the pest pressure is very high. The economic efficacy of *Trichogramma* wasp application can be estimated from EIL values given in Table 4. For a high efficacy of *Trichogramma* wasp, a high abundance of pest is the most important. *Trichogramma* wasp

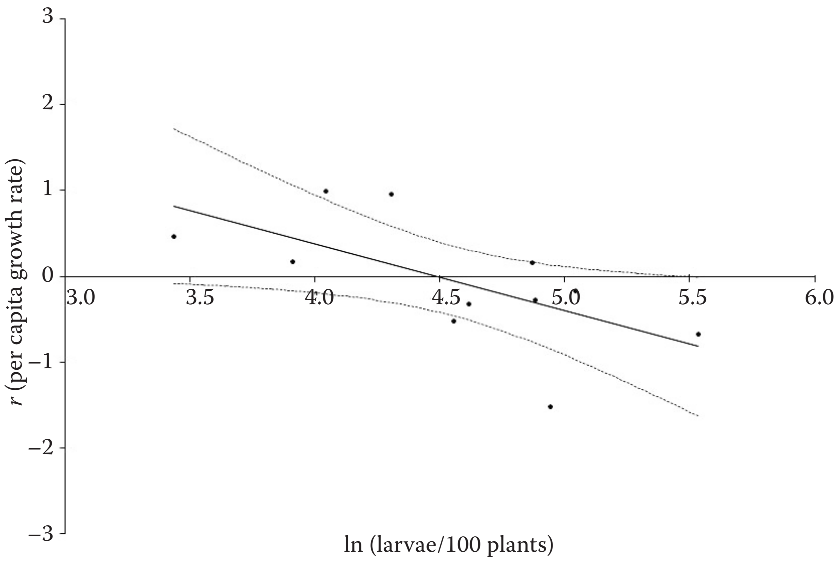

Figure 3. Regression model of densities of ECB larvae per 100 plants (x) and per capita growth rate ($r = \ln(\text{Nt}/\text{Nt} - 1)$ before adoption of Bt maize – data from Ivanovice na Hané (2002–2008), Prague (2002–2004), and Čáslav (2005–2008)

is suitable means of the maize protection against ECB in organic farming.

Damage curve of ECB and economic injury level

The ECB in the infested areas in Europe occurs in a large proportion of fields ranging from 20% in Hungary to 60% in Spain, and the estimated yield losses between 5% and 30% are typical without control measures (Meissle 2010). In the Czech Republic, ECB occurs in 50% proportion and the estimated yield losses on the respective area ranges from 10% to 15%.

According to the definition of the economic injury level (Pedigo *et al.* 1986), we calculated the economic injury level as the population density of ECB corresponding to the larval stalk tunnelling before harvest causing economic damage. The economic injury level definition is the notion that a given number of pests is an index of the total injury caused by ECB. We used the insect number as an index of the total injury from ECB. The number of tunnels in plant before harvest corresponds to the abundance of pest in autumn (number of larvae finishing the development). For the calculation of the damage curve and EIL, we used the numbers of tunnels in the stalks and ears. Hurley *et al.* (2004) used a mathematical model for the conversion of average tunnelling (cm/plant) by ECB to the abundance of larval population. Both methods provide comparable values of ECB abundance.

The damage curve of ECB was quantified based on the experimental data from the field trials (Table 2). A relatively low value of R-squared is higher than that in a similar model developed by Hurley *et al.* (2004). According to Hurley *et al.* (2004), the low correlation between the tunneling and yield loss resulted in alow adjusted R-squared that is typical with ECB field data. According to the damage curve, it is possible to estimate the percentage decrease of the grain yield caused by one ECB larva. According to our model, one ECB larva that finished the development in maize causes a decrease of yield by 7.9%. Bode and Calvin (1990) determined the yield loss relationship and economic injury level of ECB population infesting field maize. According to their data, the average grain weight reduction over years was 6% and 5% per larva when the stalk feeding was initiated during the 10-leaf and 16-leaf stages of the plant development. The experimental data by Bode and Calvin (1990) correspond very well with those from our regression model of the damage curve.

The damage curve determined in our study is a simple model for the estimation of grain yield losses ain caused by ECB. This model is usable in the conditions of Central Europe. More sophisticated models include hierarchical models for the estimation of the yield losses caused by ECB that were used by Hutchison *et al.* (2010). Mean yield losses at a regional level were estimated by Hutchison *et al.* (2010) using the observed ECB population densities and estimated models of larval stalk tunnelling and the associated yield losses after the models of Mitchell *et al.* (2002) and Hurley *et al.* (2004).

We modified the theoretical model for the determination of the economic injury level according to Pedigo *et al.* (1986) (see Material and Methods) to enable the calculation of the economic injury level according to the damage curve and concrete economic and agronomic parameters. The original modification of the model enables also to include the biological efficacy of the control measures used into calculation.

The parameters of the regression model for the growth rate and monitoring of the abundance of ECB for Central Europe are similar to those of the regression model for Illinois in the USA before the adoption of Bt maize as published by Hutchison *et al.* (2010). In Illinois, the mean density of ECB was reduced by 64% after 40% adoption of Bt maize. We can use our model for the growth rate of ECB as a baseline for the estimation of the population density after adoption of Bt maize in Central Europe for future analyses.

Hutchison *et al.* (2010) documented high benefits of Bt maize growing in the USA over 14 years of growing high proportions of Bt maize. They showed that the pest suppression is directly associated with the use of transgenic maize. Their findings indicate that the economic benefits accrue not only to farmers planting Bt maize, but also to those planting non-Bt maize as a result of area wide pest suppression, and these suppression benefits can be equal or exceed the benefits to Bt maize growers. The benefit to farmers due to Bt maize growing increases with the increasing proportion of Bt maize in a given state in the USA (in wider region). The suppression of pest on non-Bt plant area near Bt plant, so area called halo effect was described by Tabashnik (2010). The females of ECB lay eggs indiscriminately on Bt and non-Bt maize and the larvae hatching on Bt maize die. The regional pest population of ECB can be greatly reduced, resulting in lower damage on non-Bt maize. Instead of the propagation of Bt maize in Central Europe and utilisation of the halo effect, the area of fields treated with synthetic insecticides is increasing (State Phytosanitary Administration, unpublished data).

Management in refuge

The prevention of the development of ECB resistance to Bt toxin has currently two key components: (1) using Bt maize hybrids with Bt toxin expression in plant tissues in high concentration, (2) planting refuge areas of non-Bt maize. This so-called "high dose/refuge" strategy ensures the killing of the most resistant ECB and enables to mate the resistant ECB from Bt maize area with the predominantly susceptible ECB from the non-Bt refuge (Hyde *et al*. 2000). The refuges promote the surviving susceptible insects to mate with the resistant insects that survive on Bt maize. Farmers have to grow 20% of non-Bt maize as refuge. However, at a low proportion of Bt maize in the region, the damage caused by ECB in the refuges can be very high. Hence, the treatment of refuges with insecticides or *Trichogramma* wasp is recommended in Europe to prevent the damage. The economic efficacy of the refuges treatment can be also evaluated according to the particular economic and agronomic parameters in accordance with the determination of the economic injury level values given in Table 4.

CONCLUSIONS

The results of perennial field trials with Bt maize MON 810 in the Czech Republic show a high abundance of ECB in grain maize in the first decade of this century. The abundance and damage level by ECB are comparable to the abundance and damage level in Illinois in the USA before the adoption of Bt maize (Hutchison *et al*. 2010). The abundance of ECB exceeding the determined economic injury levels on more than 50% of 100 000 ha area of grain maize in the Czech Republic and using any control measure was economically effective. Up to 2010, the use of synthetic insecticides, including selective pesticides for the control of maize against ECB, prevailed on more than 60% of the growing area. However, Bt maize (MON 810) use stagnated at only 5–8% of the production area. The reasons for the stagnation of Bt maize use in the Czech Republic are higher administrative demands in comparison to the conventional maize hybrids, and the problems with the GMO products marketing within the EU framework. Political efforts are made in the EU to reduce the pesticide use and to increase the implementation of integrated pest management. Bt maize is one of the tools in the integrated pest management, a highly specific and highly efficient pest control measure that allows the growers to produce high-quality grain (Meissle 2011). The discrepancy between the effort to reduce pesticides used and the restriction of growing GMO based on scientifically unfounded fears of society is evident in the contemporary Europe.

The evaluated efficacy of Bt maize against ECB and the determined economic injury level by ECB on non-Bt maize before the adoption of Bt maize enable the calculation of yield losses in the dependence on the EBC population density. The evaluation of the *Trichogramma* wasp efficacy and evaluation of selective insecticides eficcacy and the determined damage curve enable to model the values of economic injury level of ECB for particular economic and agronomic conditions, and also enable evaluate the economic efficacy of the control measures with regard to their different biological efficacy. Based on the results of field trials and based on economic injury level modelling, a higher economic efficacy of Bt maize growing in comparison with other control measures such as selective insecticides or biological control with *Trichogramma* wasp is documented in this study. Growing of Bt maize in Central Europe has verifiable economical benefits as compared to conventional technologies and the use of insecticides in the pest control. Growing of Bt maize has no important negative effect on the environment, biodiversity, or soil fertility (Frouz *et al*. 2008), in contrast to the negative effects of synthetic pesticides. The knowledge about the abundance of ECB in non-Bt maize and the use of a regression model to forecast the growth rates of ECB should enable the estimation of possible benefits after the adoption of Bt maize on larger areas and at various proportions of Bt maize growing in Central Europe.

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