Ostrinia nubilalis parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*)

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In this study, we evaluated in field trials the effects on non-target species, of transgenic corn producing the Cry1Ab toxin of *Bacillus thuringiensis* (*Bt*). In 1998, we collected *Ostrinia nubilalis* (Hübner) larvae from transgenic *Bt* corn (Novartis Hybrid 176) and non-*Bt* corn at four geographical sites. We found a significant variation in parasitism by the tachinids *Lydella thompsoni* (Herting) and *Pseudoperichaeta nigrolineata* (Walker) among sites, and more parasitism in non-*Bt* than in *Bt* fields. The *Bt* effect did not vary significantly among fields. In 1999, we performed a field experiment at two sites, comparing the temporal abundance of non-target arthropods in *Bt* corn (Monsanto Hybrid MON810) and non-*Bt* corn. The non-target insects studied included the aphids *Metopolophium dirhodum* (Walker), *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.), the bug *Orius insidiosus* (Say), the syrphid *Syrphus corollae* (Meigen), the ladybird *Coccinella septempunctata* (L.), the lacewing *Chrysoperla carnea* (Stephens), thrips and hymenopteran parasitoids. For all species but one, the number of individuals varied greatly over the season but did not differ between the types of corn. The only exception was thrips which, at one site, was significantly more abundant in *Bt* corn than in non-*Bt* corn. However this difference did not remain significant when we took the multiple tests into account. Implications for pest resistance management, population dynamics and risk assessment are discussed.

Key words: *Ostrinia nubilalis*, *Lydella thompsoni*, *Pseudoperichaeta nigrolineata*, *Metopolophium dirhodum*, *Rhopalosiphum padi*, *Sitobion avenae*, *Orius insidiosus*, *Syrphus corollae*, *Coccinella septempunctata*, *Chrysoperla carnea*, *Bacillus thuringiensis*, non-target insects, Aphididae, predators, parasitoids, transgenic corn.

INTRODUCTION

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Ledipdoptera: Crambidae), is the major lepidopteran pest of corn (*Zea mays* (L.)) and is responsible for major yield losses in the USA and Europe (Krattiger, 1997). Corn plants genetically transformed to produce the Cry1Ab, Cry1Ac and Cry9C endotoxins of *Bacillus thuringiensis* (referred to as *Bt* corn) have been developed and are currently planted over substantial areas, particularly in the United States (Hilder and Boulter, 1999; James, 2000).

The main risk with the cultivation of *Bt* corn is the selection of resistance to *Bt* toxins in ECB populations (Gould, 1998; Hails, 2000). Strains resistant to Cry1Ab and Cry1Ac toxins have already been selected in

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Table 1. Comparative level of parasitism of ECB larvae by the tachinids *L. thompsoni* and *P. nigrolineata* in adjacent *Bt* and non-*Bt* corn fields at four experimental sites.

| | Midi-Pyrénées region | | | | | | Rhône-Alpes region | | All sites | |
| | Bérat | | Rieumes | | Lavelanet | | Salaise-sur-Sanne | | | |
	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*	*Bt*
Corn stalks observed	100	200	106	100	250	250	255	561	711	1 261
Larvae per stalk	5.25	1.83	1.58	0.81	1.89	1.05	1.73	1.38	2.26	1.18
% parasitism	6.29	0.55	0.60	0	2.75	0.76	3.17	0.26	3.80	0.40

laboratory conditions (Huang et al., 1997; Bolin et al., 1999; Chaufaux et al., 2001), but there are as yet no reports of resistance occurring in the field. Moreover, the frequency of alleles conferring resistance to *Bt* corn may be low enough to be managed (Andow et al., 2000; Bourguet et al., submitted manuscript). Toxin resistance in the ECB populations may not be the only risk when using *Bt* corn on a large scale (Hilder and Boulter, 1999; Hails, 2000). Although the Cry proteins produced in transgenic corn are considered to be specific (MacIntosh et al., 1990), some side effects of these toxins on non-target species have been reported (Salama et al., 1982; Flexner et al., 1986; Hilbeck et al., 1998, 1999; Losey et al., 1999).

Adverse effects of *Bt* corn on non-target insects may arise due to several factors: high concentrations of *Bt* toxin in corn pollen, changes in the *cry1Ab* gene resulting in the production of protein variants in different hybrids (Jepson et al., 1994). In addition, *Bt* protein is produced throughout the growing season whereas *Bt* sprays degrade very quickly in the field. Few studies have evaluated the effect of transgenic corn on non-target species directly in the field (Orr and Landis, 1997; Pilcher et al., 1997; Lozzia, 1999).

This study was undertaken to obtain scientific data for the French *Comité de Biovigilance*, a consultative committee for the assessment of environmental risk following the introduction of genetically modified organisms. This study had two goals: (i) to compare the level of parasitism of corn borer larvae collected from *Bt* and non-*Bt* corn plants; and (ii) to compare the temporal occurrence and field abundance of several non-target insects on transgenic *Bt* corn and on untransformed non-*Bt* corn hybrid.

RESULTS

Tachinid parasitism

Only stalks and ears infested with *O. nubilalis* were chosen, so larval density cannot be strictly compared between transgenic and non-transgenic corn. However, at all sites, there were fewer larvae per stalk on *Bt* corn than on non-*Bt* corn (Tab. 1).

We collected enough larvae from Hybrid 176 for a comparison between corn types to be made. ECB may have been present in *Bt* corn for two main reasons. First, the expression level may have been too low to control ECB as expected. Second, the ECB collected from transgenic stalks may have had a low susceptibility to the Cry1Ab toxin. This second possibility can be ruled out for several reasons. First, *Bt* sprays have never been used on corn fields in France, so there was no opportunity for the selection of resistance alleles before the introduction of *Bt* corn. Second, offspring from the fifth instar collected from both *Bt* and non-*Bt* corn did not differ in susceptibility to the Cry1Ab toxin in laboratory bioassays (Chaufaux and Bourguet unpublished results). Finally, isofemale lines established from ECB captured in the vicinity of these *Bt* corn fields did not demonstrate the existence of resistant ECB (Bourguet et al., submitted manuscript). Conversely, it is known that during the latter part of the field season, the concentration of *Bt* toxin declines in transgenic corn. Indeed, the production of Cry1Ab toxin by Hybrid 176 has frequently proved insufficient for the control of subsequent generations of ECB (Walker et al., 2000). Thus, in the Midi-Pyrénées and Rhône-Alpes regions, which have two generations of ECB per year (Anglade, 1970), the level of Cry1Ab toxin in the Novartis 176 Hybrid, Furio Cb[®] and Occitan Cb[®], was probably lower than required to kill the fifth instars collected from the stalks and ears at the time at which they hatched as first instars.

At all sites, the level of parasitism of ECB larvae was lower in *Bt* corn than in non-*Bt* corn (Tab.1). A log linear contingency table analysis show that there was a significant variation in parasitism among sites ($P = 0.0044$, see Tab. 2) and more parasitism by tachinids in non-*Bt* corn fields than in *Bt* corn fields ($P < 0.0001$, see Tab. 2). Moreover, the *Bt* effect does not vary significantly among fields ($P = 0.6585$, see Tab. 2).

Table 2. Log-linear contingency table analysis testing the effect of site, type of corn (*Bt* versus non-*Bt* corn) and the interaction site × type of corn on the number of larvae parasitized by tachinids.

Source	d.f.	Chi-Square	$P >$ ChiSq
Site	3	13.10	0.0044
Type of corn	1	26.80	< 0.0001
Site × type of corn	3	1.60	0.6585

Aphids and their enemies

For each species, the mean number of individuals found on each date is reported in Figures 1 and 2 for Aussonne and Montesquieu-Lauragais, respectively. At both experimental sites, and on each date, no significant differences (*t*-tests, $P > 0.05$) were observed in the numbers of each species of aphid (*Metopolophium dirhodum* (Walker), *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.)), or of its enemies (the ladybird *Coccinella septempunctata,* the syrphid *Syrphus corollae* (Meigen), the lacewing *Chrysoperla carnea* (Stephens), the bug *Orius insidiosus* (Say) and thrips and hymenopteran parasitoids). Most species displayed a progressive increase in number, reaching a maximum between mid-June and mid-July. Interestingly, there was a lag between the appearance of aphids and that of their predators; aphids were mostly found early in the season, whereas predators appeared later. This was particularly true in Aussonne, where the three species of aphids were collected only in June whereas predators were found from mid-June to the end of July.

The results of the ANOVA are given in Table 3. For both sites, all species studied were strongly influenced by sampling time (*P* generally < 0.0001 and always < 0.05) but temporal abundance did not differ significantly between the two types of corn (*Bt* versus non-*Bt* corn) except for thrips, which was more abundant in *Bt* corn throughout the season at the Aussonne site ($F_{1,3} = 15.82$; $P = 0.028$). However, this difference was not significant when we took the multiple tests into account (Bonferroni test) (Holm, 1979). There was no significant interaction between sampling time and type of corn ($P > 0.50$).

Table 4 gives the mean number of individuals (and its 95% CI interval) observed on 10 consecutive plants in *Bt* and non-*Bt* corn fields over the season. Only the total number of thrips was significantly higher (*t*-test, $t_s = 4.34$; df = 6; $P < 0.005$) in the *Bt* corn plots (153.3 ± 9.5) than in the non-*Bt* corn plots (99.0 ± 23.2) at the Aussonne site.

DISCUSSION

We found that larvae collected from *Bt* corn (Novartis Hybrid 176) displayed a lower level of parasitism by the tachinids *Lydella thompsoni* (Herting) and *Pseudoperichaeta nigrolineata* (Walker) than did larvae collected from non-*Bt* corn. In a second experiment, we found that the abundance of several non-target arthropods (several aphids and their predators) did not differ significantly between *Bt* corn (Monsanto Hybrid MON810) and non-*Bt* corn.

Lower level of ECB parasitism

Potential explanations

Based on the review by Schuler et al. (1999) there are several possible explanations for the lower level of parasitism of ECB larvae in *Bt* corn. Some are related to the toxic effects of transgenic *Bt* corn on ECB larvae. A direct effect of Cry1Ab toxin on tachinids seems unlikely since Cry1 toxins are specific for insect species of the order Lepidoptera (MacIntosh et al., 1990). However, the sublethal doses of toxin produced by Furio Cb® and Occitan Cb® transgenic corn may have weakened ECB larvae so that the larval parasitoid has been affected indirectly (Herzog and Funderburk, 1985; Lopez and Ferro, 1995; Van Emden, 1995). Whereas ECB larvae in non-*Bt* corn fields were ideal for parasitization by tachinids, ECB larvae in *Bt* corn were probably smaller and may not have been suitable for parasitization.

One of the most obvious way in which transgenic corn may have affected the level of tachinid parasitism is by decreasing density of ECB larvae. In corn plots, the parasitism of *O. nubilalis* larvae by the ichneumonid *Eriborus terebrans* (Gravenhorst) was found to be positively correlated with host density (number of larvae per plant) in the corn broadleaf weed community (Puvuk and Stinner, 1992). Although Onstad et al. (1991) showed that parasitism by the braconid *Macrocentrus grandii* (Goidanich) was not density dependent, they reported a significant correlation between the proportion of larvae that were parasitized and the frequency of stalks infested with *O. nubilalis*. At the four sites investigated in the present study, the frequency of stalks infested with *O. nubilalis* would have been much greater in the non-*Bt* corn fields than in the *Bt* corn fields, therefore theoretically favoring parasitism in the non-*Bt* fields.
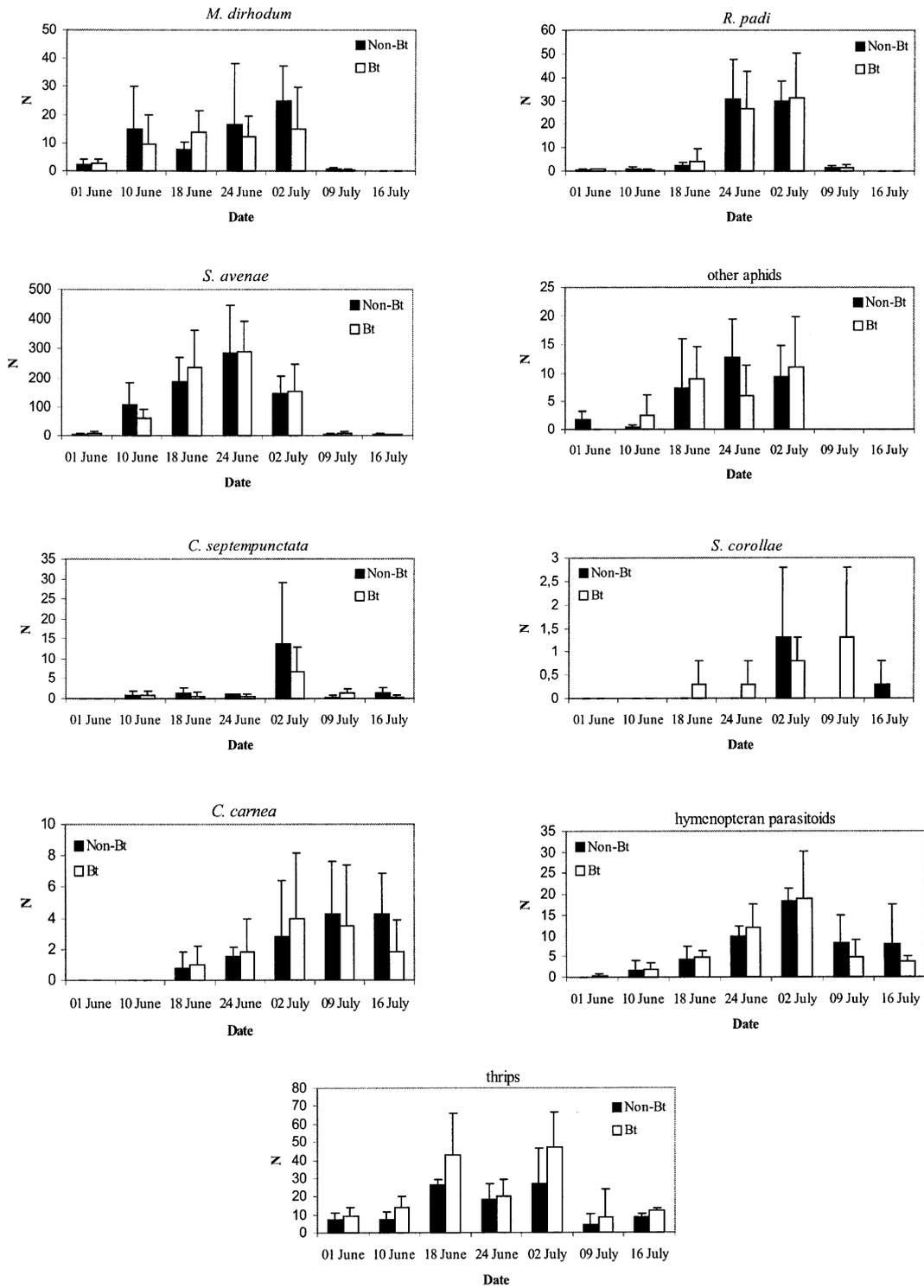
Figure 1. Number (mean of the replicates ± standard deviation) of each species of aphids, predators and thrips per 10 corn plants and per date in *Bt* corn and non-*Bt* corn fields in Aussonne.
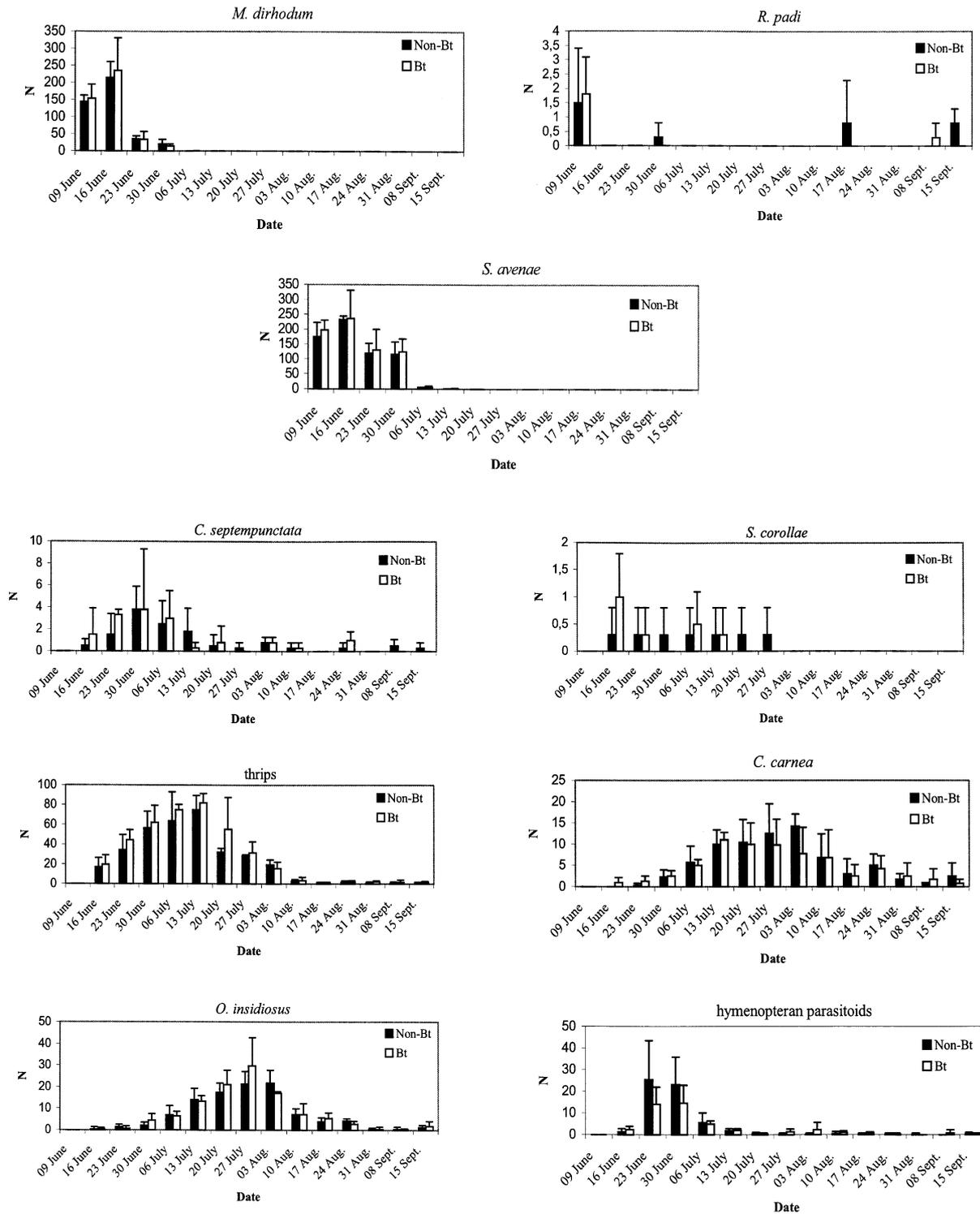
Figure 2. Number (mean of the replicates ± standard deviation) of each species of aphids, predators and thrips per 10 corn plants and per date in *Bt* corn and non-*Bt* corn fields in Montesquieu-Lauragais.

Table 3. ANOVA testing the effect of type of corn, the sampling date and the interaction sampling date × type of corn on the number of aphids (A), predators and thrips (B) at the two experimental sites.
A. Aphids.

	d.f.	*R. padi*			*M. dirhodum*			*S. avenae*			Other Aphids		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P
Montesquieu-Lauragais													
Level 1													
Type of corn	1	0.075	0.090	0.783	70.53	0.065	0.816	496.13	0.581	0.501	-	-	-
Level 1 error: Type of corn × Replicate	3	0.831	-	-	1091.5	-	-	854.11	-	-	-	-	-
Level 2													
Sampling date	14	1.401	5.407	< 0.0001	35637	80.48	< 0.0001	52819	69.46	< 0.0001	-	-	-
Sampling date × Type of corn	14	0.111	0.427	0.962	73.30	0.166	1.000	117.69	0.155	1.000	-	-	-
Level 2 error	84	0.259	-	-	442.80	-	-	760.50	-	-	-	-	-
Aussonne													
Level 1													
Type of corn	1	0.875	0.040	0.854	56.00	2.551	0.209	117.16	0.057	0.831	2.161	0.236	0.661
Level 1 error: Type of corn × Replicate	3	21.78	-	-	21.95	-	-	2040.4	-	-	9.161	-	-
Level 2													
Sampling date	6	1558.5	25.23	< 0.0001	471.91	6.729	< 0.0001	103846	19.73	< 0.0001	175.44	8.602	< 0.0001
Sampling date × Type of corn	6	6.333	0.080	0.996	52.21	0.520	0.618	1558.7	0.260	0.935	19.58	0.960	0.466
Level 2 error	36	61.78	-	-	70.13	-	-	5262.7	-	-	20.39	-	-

Implications for population dynamics

More than 200 crop and weed species are colonized by *O. nubilalis* (Lewis, 1975), one of the best alternative hosts being the common mugwort, *Artemisia vulgaris* (L.) which is common over more than 80% of Europe (Thompson and Parker, 1928). All tachinids found in ECB larvae collected from corn have also been found in larvae collected from mugwort (Thompson and Parker, 1928). Finally, tachinids such as *L. thompsoni* are known to parasitize larvae of several other lepidopteran species including the noctuids *Archanara geminipuncta* (Haw) and *Archanara dissoluta* (Treitschke) (Galichet, 1986). Therefore, even if the number of tachinid parasitoids declines due to ECB depletion in *Bt* corn, their persistence is probably not threatened.

Implications for pest resistance management

One of the main risks in the cultivation of *Bt* toxin-producing plants is the selection of resistance alleles in the target pest species (Tabashnik, 1994; Gould, 1998). The high dose/refuge strategy developed by Alstad and Andow (1995), is considered to be the most appropriate for managing such resistance and is currently used for *Bt* cotton and *Bt* corn in North America (Ostlie et al., 1997). For the high dose/refuge strategy to be effective, only resistant homozygous individuals (RR) should survive on *Bt* crops (Alstad and Andow, 1995). To achieve this goal, the Environmental Protection Agency (EPA) has defined a high dose as 25 times the concentration needed to kill susceptible larvae (EPA, 1998). The results reported here indicate that, later in the growing season, Hybrid 176

Table 3. (continued).
B. Predators and thrips

	d.f.	*C. septempunctata*			*S. corollae*			*C. carnea*			*O. insidiosus*			*Thrips*			*Hymenopteran*		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Montesquieu-Lauragais																			
Level 1																			
Type of corn	1	0.408	0.344	0.599	0.033	0.059	0.824	10.21	0.490	0.535	9.075	0.486	0.536	529.20	2.289	0.228	31.01	0.641	0.482
Level 1 error: Type of corn × Replicate	3	1.186	-	-	0.567	-	-	20.85	-	-	18.68	-	-	231.18	-	-	48.39	-	-
Level 2																			
Sampling date	14	10.50	4.830	<0.0001	0.276	2.762	0.002	138.13	11.64	<0.0001	548.37	39.70	<0.0001	6042.4	53.29	<0.0001	339.19	15.66	<0.0001
Sampling date × Type of corn	14	1.123	0.517	0.917	0.105	1.048	0.416	7.655	0.645	0.819	16.68	1.208	0.285	89.25	0.787	0.680	27.31	1.261	0.249
Level 2 error	84	2.173	-	-	0.100	-	-	11.86	-	-	13.81	-	-	113.38	-	-	21.66	-	-
Aussonne																			
Level 1																			
Type of corn	1	19.45	0.725	0.457	0.286	6.000	0.092	0.071	0.030	0.874	-	-	-	840.88	15.82	0.028	2.571	0.085	0.790
Level 1 error: Type of corn × Replicate	3	26.83	-	-	0.048	-	-	2.405	-	-	-	-	-	53.161	-	-	30.33	-	-
Level 2																			
Sampling date	6	106.1	5.463	0.000	1.155	2.867	0.022	16.95	3.108	0.015	-	-	-	1308.6	9.132	<0.0001	317.00	12.64	<0.0001
Sampling date × Type of corn	6	14.03	0.722	0.634	0.619	1.537	0.194	1.321	0.242	0.959	-	-	-	115.92	0.809	0.570	8.821	0.352	0.904
Level 2 error	36	19.42	-	-	0.403	-	-	5.454	-	-	-	-	-	143.30	-	-	25.09	-	-

Table 4. Number (mean of the replicates ± standard deviation and 95% CI for mean) of aphids and predators and thrips observed on 10 corn plants over the season in *Bt* corn and non-*Bt* corn fields at the two experimental sites.

| | Aussonne | | | | Montesquieu-Lauragais | | | |
| | non-*Bt* | | *Bt* | | non-*Bt* | | *Bt* | |
	Mean ± SD	95% CI for mean	Mean ± SD	95% CI for mean	Mean ± SD	95% CI for mean	Mean ± SD	95% CI for mean
Aphids:								
R. padi	66.3 ± 24.2	27.1–104.7	64.5 ± 38.4	3.4–125.6	2.8 ± 3.6	0–8.5	2.0 ± 1.4	0–4.3
M. dirhodum	67.0 ± 45.8	0–139.9	53.0 ± 36.0	0–110.3	415.8 ± 65.2	312.1–519.4	438.8 ± 145.0	208.0–669.5
S. avenae	738.5 ± 292.1	273.7–1203.3	758.8 ± 156.2	510.2–1007.3	648.5 ± 59.4	553.9–743.1	709.5 ± 102.1	547.0–872.0
Other aphids	31.3 ± 14.5	8.2–54.3	28.5 ± 14.7	5.1–51.9	-	-	-	-
Predators:								
C. septempunctata	18.3 ± 16.5	0–44.6	10.0 ± 3.8	3.9–16.1	13.0 ± 4.4	6.0–20.0	14.8 ± 6.7	4.2–25.3
S. corollae	1.5 ± 1.7	0–4.3	2.5 ± 1.3	0.4–4.6	1.8 ± 2.4	0–5.5	2.3 ± 2.2	0–5.8
C. carnea	11.5 ± 7.5	0–23.4	12.0 ± 2.2	8.6–15.4	75.5 ± 12.2	56.2–94.8	66.8 ± 15.1	42.7–90.8
O. insidiosus	-	-	-	-	103.0 ± 18.7	73.3–132.7	111.3 ± 8.6	97.5–125.0
Hymenopteran parasitoids	50.5 ± 10.1	34.5–66.5	47.5 ± 14.5	24.5–70.5	59.5 ± 28.7	13.8–105.2	44.3 ± 13.3	23.2–65.3
Thrips	99.0 ± 23.2	62.1–135.9	153.3 ± 9.5	138.2–168.3	311.3 ± 87.8	171.5–451.0	373.8 ± 24.1	292.4–455.1

Bt corn, Furio Cb® and Occitan Cb® do not produce the toxin at a concentration high enough to control all SS individuals.

In transgenic corn producing high doses of toxin, any surviving individuals are likely to be RR. Our results suggest that such resistant individuals would be less likely to be parasitized by tachinid flies, which is not favorable for pest resistance management.

Aphids and their enemies

We are aware of only three other studies evaluating the adverse effect of *Bt* corn on non-target species. Pilcher et al. (1997) found no detrimental effects on predators such as ladybirds, anthocorids and chrysopids in *Bt* corn in two years of field evaluations. Orr and Landis (1997) also found no significant differences in the numbers of aphid and *O. nubilalis* predators between transgenic and isogenic plots. The last survey, performed by Lozzia (1999), included a wide variety of arthropods: Aphididae, Cicadellidae, Aranea and Coleoptera (Chrysomelidae, Coccinelidae, Staphilinidea) and other insects living in close contact with these or *O. nubilalis*, such as hymenopteran parasitoids and members of the Diptera Syrphidae. He also found no significant difference in the abundance of non-target insects in isogenic and transgenic *Bt* corn during 1997 and 1998.

Our results are consistent with the notion that transgenic *Bt* corn has no drastic toxic effect on non-target species. Only the abundance of thrips was affected, at one experimental site: at Aussonne more thrips were found in *Bt* corn plots than in non-transgenic corn plots, throughout the season. However, this pattern may result from an artifact due to the large number of comparisons performed and, therefore, may not have been genuine; the difference was not significant following Bonferroni correction for multiple tests. This point requires further investigation.

The fact that we were unable to reject the null hypothesis does not mean that transgenic hybrid corn does not have an acute effect on the species studied. Non-significant results do not necessary imply that there was no effect. Indeed, due to high variability in the number of species between replicates and/or the low density of some non-target species (e.g. *C. septempunctata* and *S. corollae*), we were unable to detect small differences in the number of individuals collected in the two types of corn over the season.

The future development of transgenic cultivars may depend on the potential risks and benefits of genetically modified organisms. One of the first issues that needs to be addressed is: what constitutes an ecological risk? One broad definition (Hails, 2000) is that an ecological threat might be posed if the presence of a plant containing a transgene has a negative impact on the ecosystem,
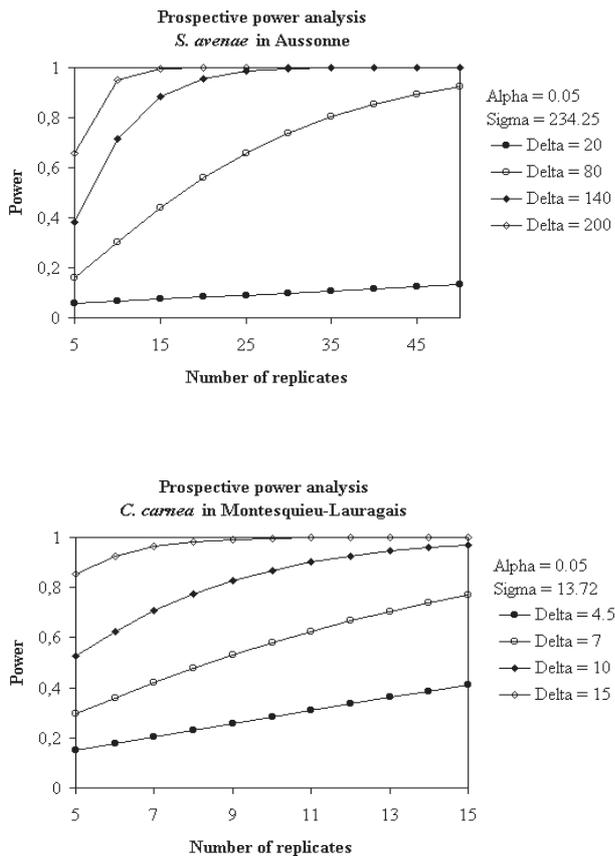
Prospective power analysis
S. avenae in Aussonne

Alpha = 0.05
Sigma = 234.25
— ● — Delta = 20
— ○ — Delta = 80
— ◆ — Delta = 140
— ◇ — Delta = 200

Prospective power analysis
C. carnea in Montesquieu-Lauragais

Alpha = 0.05
Sigma = 13.72
— ● — Delta = 4.5
— ○ — Delta = 7
— ◆ — Delta = 10
— ◇ — Delta = 15

Figure 3. Prospective power analysis for future studies. The power tells how likely the experiment is to detect a difference (here the difference in the number of individuals given by the delta value), at a given alpha level. Power is plotted as a function of the number of *Bt* and non-*Bt* plot replicates for various delta values. These projections were performed using estimates of standard deviation of the error (sigma) calculated from the collected data reported in Table 4. Power was calculated for a significance level alpha = 0.05.

season may not detect small effects of *Bt* corn unless we increase the number of replicates or the number of corn plants on which insects are counted. Our estimates of variance can be used for a prospective power analysis for some undetermined future studies. This is illustrated in Figure 3 for the aphid *S. avenae* and the lacewing *C. carnea* at Aussonne and Montesquieu-Lauragais sites, respectively, where the power is plotted as a function of the number of replicates for different delta values. Figure 3 shows that if the variance estimates from our samples are representative of what the next study will find, that study will require a high number of replicates to have sufficient power (i.e. > 0.8) in detecting small differences between the number of individuals found in *Bt* corn fields and that in non-*Bt* corn fields. For example, at Aussonne a difference of 80 aphids observed on 10 corn plants over the season (a difference ~10%) between the two types of corn would require no less than 40 replicates (instead of 4 performed in the present study) to give a 80% likelihood to reject the null hypothesis at a alpha level of 0.05 and conclude that the type of corn does actually affect the number of this species of aphid (see Fig. 3). Thus, given the effort required to perform such experiments, it is probably unrealistic to expect to detect a difference of 10–20% in the abundance of most non-target species.

Three conclusions may be drawn from this. One concerns the detection of such small effects in the field. Although not detectable over a single season, these effects may be cumulative over time. Further research is required to determine the type of survey needed to detect such cumulative effects. The second conclusion concerns the ecological importance of small variations in non-target species. As we cannot conclude that *Bt* corn has no effect on non-target species (we cannot detect variation in abundance of the order of one or a few percent), it would be worthwhile defining a threshold below which any effect can be considered. The last conclusion concerns the species under investigation. Theoretically, each transgenic plant (and perhaps each transformation event) may have a specific effect on non-target species. In a realistic risk assessment protocol, only a fraction of the non-target species can be considered. The choice of species for study is a major challenge for further research on the environmental impact of transgenic plants.

MATERIALS AND METHODS

European corn borer parasitism

Four sites were studied: Rieumes, Lavelanet and Bérat (Midi-Pyrénées region, France) and Salaise-sur-Sanne

relative to the status quo. In comparison with the use of chemical insecticide treatments, *Bt* corn does not seem to present a risk to the conservation of aerial insect biodiversity. However, such chemical treatments against ECB are not the general status quo in the United States, where less than 5% of the area under corn is treated (Nelson et al., 1999). The same is true for France where only about 20% of the area under corn is chemically treated against this pest species (Riba and Chaufaux, 1998).

Risk assessment and future research

These present results have important consequences for risk assessment. Field experiments performed over one

(Rhône-Alpes region, France). At each site we studied fields planted with *Bt* corn (Novartis Hybrid 176: Furio Cb® in Midi-Pyrénées, Occitan Cb® in Rhône-Alpes) and non-*Bt* corn (Furio® in Midi-Pyrénées, Occitan® in Rhône-Alpes). We do not know the history of the varieties so that we cannot conclude on how nearly isogenic they are. Depending on the number of backcrosses performed during the breeding process, control and *Bt* varieties may be genetically different at 0.2–3% of the corn genome. At each site, the fields containing *Bt* corn and non-*Bt* corn were immediately adjacent to each other, identical in size (each plot measuring ~200 m^2 at Rieumes, Lavelanet and Bérat and ~1 ha at Salaise-sur-Sanne), planted the same day (23 April, 24 April and 7 May 1998 at Rieumes, Lavelanet and Bérat, respectively and early May at Salaise-sur-Sanne) and subjected to similar, typical cultivation practices. None of these fields was treated with chemical insecticide. To avoid edge effects from wild plant communities, plots were surrounding by fields containing non-*Bt* corn. In each site and in both types of field, fifth instar diapausing ECB larvae were randomly sampled at the same date during October 1998. Between 100 and 561 stalks and ears were examined to obtain a minimum number of ECB larvae from each type of field. We expected ECB larvae to be present in fields of *Bt* corn because both Hybrid 176 varieties (Furio Cb® and Occitan Cb®) are known to produce insufficient Cry1Ab toxin to control the second generation of ECB larvae (Walker et al., 2000; Bourguet and Chaufaux, unpublished data). Each larva was removed from the stalk or ear, brought back to the laboratory and kept at 4 °C to facilitate completion of the diapause. Termination of diapause was ensured by placing larvae on a corn diet with a photoperiod of 16 h and temperatures of 20 to 25 °C until either moths or tachinid flies emerged. The flies were mostly *L. thompsoni*, although a small proportion was identified as *P. nigrolineata* (Walker).

We investigated variations in parasitism among sites and between *Bt* and non *Bt* fields using a log-linear contingency table analysis using JMP IN 3.0 Software (SAS Institute, 1996).

Aphids and their enemies

Field experiments were performed in 1999 at two geographical sites: Montesquieu-Lauragais and Aussonne, both located in the Midi-Pyrénées region of France. Transgenic *Bt* corn (Monsanto hybrid MON 810, Elgina® variety) and non-*Bt* corn (Cecilia® variety) seeds were planted in 12-row plots, 10 by 90 m in size. Rows were planted with 0.75 m row spacing and 0.15 m plant spacing within a row. Four pairs of *Bt* and non-*Bt* plots were planted contiguously in a split-plot design and five rows of non-*Bt* corn were planted on each side of the two external plots to prevent border effects. In total, the field experiment comprised 106 rows and covered 85 by 90 m. Field experiments were subjected to normal cultivation practice with no chemical insecticide treatments.

Insects were counted weekly from 9 June 1999 to 15 September 1999 at Montesquieu-Lauragais and from 1 June 1999 to 16 July 1999 at Aussonne. For each replicate and on each date, insects were counted on a set of 10 different consecutive corn plants within the seventh row. Sampled sets on different dates were separated by 10 consecutive corn plants. All life stages of aphids and their enemies, both predators and hymenopteran parasitoids, were counted. The aphids detected were *M. dirhodum*, *R. padi*, *S. avenae* and the predators were the bug *O. insidiosus*, the syrphid *S. corollae*, the ladybird *C. septempunctata*, the lacewing *C. carnea* and several thrips species (species not determined). The hymenopteran parasitoids were mostly *Praon* sp., *Aphidius* sp., and *Aphelinus* sp.

For each field site, ANOVA was used to evaluate the effect on the number of insects for each type of corn (*Bt* versus non-*Bt*), sampling date and for sampling date × type of corn interactions. We also calculated the mean number of individuals (over the four replicate plots) over the season in *Bt* and non-*Bt* corn fields. The 95% confidence interval (CI) for mean was calculated as 95% CI for mean = (plot mean) $\pm t_{0.95} \times$ (standard error of the mean), the Student's *t* quantile taking care of the extra uncertainty that results from estimating the standard error of the mean. Standard deviations were used to estimate statistical power prospectively. Power is the probability of rejecting the null hypothesis (i.e. the type of corn does not influence the number of non-target insects) when the null hypothesis is indeed incorrect and the alternative hypothesis (i.e. the type of corn does actually affect the number of non-target insects) is correct (Sokal and Rohlf, 1981). Thus, power indicates the likelihood of detecting a difference at a given alpha (α) level in undetermined future experiments. Estimation of power was performed with JMP IN 3.0 Software (SAS Institute, 1996).

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