Resistance monitoring of field populations of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* after 5 years of Bt maize cultivation in Spain

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Abstract

Approximately 22 000 hectares (5% of the total maize growing area) of transgenic maize expressing the Cry1Ab toxin from *Bacillus thuringiensis* (Bt maize) have been planted annually in Spain since 1998. Changes in the susceptibility to Cry1Ab of Spanish populations of the Mediterranean corn borer (MCB), *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae), and the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), were assessed by annual monitoring on Bt maize fields. No increase in resistance was detected in the MCB populations from Ebro, Albacete, and Badajoz, nor in the ECB populations from Ebro and Badajoz during the period 1999–2002. The susceptibility of the MCB population from Madrid fluctuated from year to year, but a gradual trend towards higher levels of tolerance was not observed. Laboratory selection assays for eight generations yielded selected strains of MCB and ECB that were 21- and 10-fold significantly more tolerant to Cry1Ab than the corresponding unselected strains, respectively. Nevertheless, none of the field-collected or laboratory-selected larvae were able to survive on Bt maize. Considering these data, no consistent shifts in susceptibility were found for Spanish populations of MCB nor ECB after 5 years of Bt maize cultivation, but systematic field monitoring needs to be continued.

Introduction

Genetically engineered maize plants expressing δ-endotoxins from *Bacillus thuringiensis* (Bt maize) are grown commercially in six countries (USA, Canada, Argentina, Spain, South Africa, and Bulgaria), and occupied a global surface of 9.9 million ha in 2002 (James, 2002). In Spain, about 22 000 ha of Bt maize (Bt176 event, var. Compa CB, Syngenta) expressing the Cry1Ab toxin were planted for the first time in 1998. A similar surface area has been grown every year during the period 1999–2002, representing around 5% of the total maize grown area.

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) is one of the most important pests of maize throughout the USA and Europe (Mason et al., 1996). However, the Mediterranean corn borer (MCB), *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae), appears to be the most damaging pest of maize in Spain (Castañera, 1986) and other Mediterranean countries (Anglade, 1972; Melamed-Madjar & Tam, 1980). The chemical control of these two species is particularly difficult because insecticide sprays are only effective during the short period which elapses between egg hatch and the larvae boring into stems. This is especially relevant for MCB, the larvae of which tunnel through the stem from the first instar. Bt maize can effectively control these two major maize pests, at the same time reducing environmental costs associated with the use of conventional insecticides (Shelton et al., 2002).

The development of resistance in target pests to Bt plants is considered the main risk to the success of this powerful control tool. Laboratory selection assays have shown that the potential development of resistance to Bt

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toxins is widespread among insect pest species (Tabashnik, 1994; Ferré & Van Rie, 2002), including ECB (Huang et al., 1997; Bolin et al., 1999; Chaufaux et al., 2001). However, field resistance in response to the spray of Bt insecticides has only been documented in some populations of the diamondback moth, *Plutella xylostella* (L.) (Tabashnik et al., 1990). Thus far, field resistance to Bt maize has not been documented, but it is expected that large-scale planting could result in rapid selection for resistance in field populations.

The development of resistance is complex, and affected by a variety of interacting influences, such as genetic, environmental, and management factors. In Spain, both the MCB and the ECB are multivoltine, completing two to three generations per year depending on the latitude of the maize growing area (López et al., 2001). In Bt maize varieties based on the Bt176 event, such as the Compa CB which is grown in Spain, the titer of the toxin decreases after anthesis (Koziel et al., 1993). It is therefore more likely to allow the survival of a second or third generation of heterozygous resistant larvae, increasing the risk of resistance development (Onstad & Gould, 1998; Walker et al., 2000).

To maintain the effectiveness of Bt plants, it is necessary to detect changes in susceptibility through regular monitoring, and to apply resistance management strategies to prevent or delay pest adaptation. Two main strategies have been proposed to assess resistance: directly, by showing decreases in susceptibility to the toxin over time within a population; or indirectly, by showing that a population with a history of relatively high exposure to the toxin is less susceptible than conspecific populations which have had less exposure (Tabashnik, 1994). The first procedure involves establishment of the natural susceptibility of different geographical populations to Bt toxins and systematic field monitoring in Bt crops to recognize resistance when it appears. Baseline susceptibility to Bt toxins has been established for ECB populations from the USA (Siegfried et al., 1995; Huang et al., 1997; Marçon et al., 1999; Reed & Halliday, 2001), and northern Italy (Marçon et al., 1999). Accordingly, we determined the baseline susceptibility to Cry1Ab toxin for four Spanish populations of MCB (Madrid, Andalucía, Galicia, and Ebro) and two populations of ECB (Ebro and Madrid) in 1998 (González-Núñez et al., 2000).

As part of a proactive resistance monitoring program, we report on the susceptibility of Spanish populations of MCB and ECB to Cry1Ab toxin after 5 years of Bt maize cultivation in Spain. Specifically, we have: (a) assessed the level of resistance to Cry1Ab of field populations during the period 1999–2002 by regular monitoring of corn borer larvae on Bt maize; and (b) performed laboratory selection of MCB and ECB strains with Cry1Ab toxin for eight generations.

Materials and methods

Insect collection

In order to monitor changes in the susceptibility of field populations during the period 1999–2002, we collected about 300–500 larvae of MCB and/or ECB at two or three commercial Bt maize fields in four major Spanish maize growing regions: Madrid, Ebro, Badajoz, and Albacete (Figure 1). Since Compa CB expresses sublethal doses of the toxin at the end of the season, larvae of the two species were found by dissecting maize stalks before harvesting. In addition, we maintained a laboratory culture of MCB established with larvae collected from Andalucía, Madrid, Ebro, and Galicia in 1998 (González-Núñez et al., 2000), and a laboratory culture of ECB initiated with specimens from Andalucía and Badajoz in 2000.

Insect culture

Field-collected larvae were dipped in a solution of 0.01% phenylmercuric nitrate to prevent pathogen contamination, and placed in plastic boxes ($21 \times 16 \times 4$ cm) (50 MCB larvae and 100 ECB larvae per box, respectively). A meridic diet, modified from Poitout & Bues (1970) by the addition of 1.6 g Wesson's salt mixture, 1.5 g Nosapiol-B (bicyclohexylamonium 2%) (Kessler Ibérica, S.L), 1 g methyl p-hydroxybenzoate, and 0.6 g aureomycin (chlortetracycline hydrochloride 5.5%) (Cyanamid Ibérica S.A) per l of diet, was prepared to feed the MCB larvae. Similarly, ECB larvae were fed a meridic diet modified from Poitout & Bues (1970) by the addition of 1.5 g methyl p-hydroxybenzoate, 1.5 g Nosapiol-B, 1.2 g sorbic acid, 0.6 g aureomycin, 9 g ascorbic acid, and 3 g benzoic acid per l of diet. Most of the larvae collected were in diapause, or entered diapause once they had reached the last instar when placed in a growth chamber (Sanyo MLR-350 H.

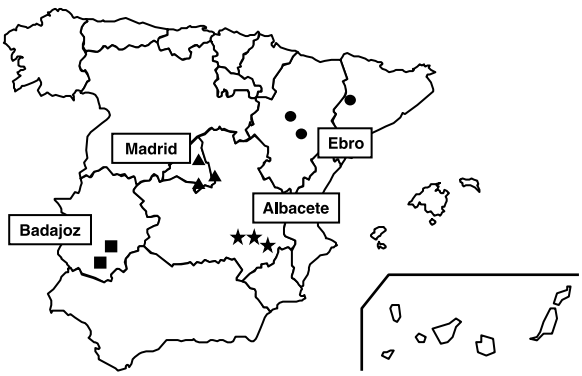

Figure 1 Spanish geographical areas where *S. nonagriodes* and *O. nubilalis* larvae were collected. Each symbol represents a sampling site (★: Albacete; ■: Badajoz; ●: Ebro; ▲: Madrid).

Sanyo, Japan) at 12 ± 0.3 °C, 70 ± 5% r.h., and a photoperiod of L12:D12. Diapausing MCB larvae were maintained in cages containing vermiculite and meridic diet, whereas diapausing ECB larvae were kept in cages containing corrugated cardboard. When required, diapause was disrupted by placing larvae at 28 ± 0.3 °C, 70 ± 5% r.h., and continuous light. To facilitate pupation of the ECB larvae, the cardboard was moistened daily.

Egg masses of MCB were obtained by confining a minimum of 100 pairs (batches of five pairs) of adults in ventilated plastic cylinders (12 cm diameter, 30 cm high) containing 5–7 maize seedlings for oviposition. Egg masses of ECB were obtained by confining a minimum of 100 pairs (batches of 25 pairs) of adults in thin wire netting cylinders (12 cm diameter, 13 cm high) confined by a cylindrical plastic box at the bottom and a waxed paper sheet at the top, where the females laid their eggs. Cotton soaked with a solution of 10% honey in water was placed in the oviposition cages for feeding, and the egg masses were removed and placed in plastic boxes provided with moistened filter paper until eclosion. Environmental conditions for mating, oviposition, and egg incubation were 25 ± 0.3 °C, 70 ± 5% r.h., and a photoperiod of L16:D8.

Bt toxin

Cry1Ab crystals (81% purity), isolated from a bacterial culture of *B. thuringiensis* ssp. *kurstaki* HD1-9 strain that produces only the Cry1Ab protein (Minnich & Aronson, 1984; Carlton & González, 1985), were provided by Syngenta.

Bioassays

The susceptibility of larvae from the first laboratory generation of field-collected populations to Cry1Ab toxin was determined as described by González-Núñez et al. (2000). The lyophilized powder was resuspended in 0.1% (v/v) Triton X-100, and 50 µl of suspension at nine different protoxin concentrations (0.75, 1.5, 3, 6, 9, 18, 24, 36, and 48 ng Cry1Ab/cm^2 for ECB, and 1.5, 3, 6, 12, 24, 36, 48, 60, and 90 ng Cry1Ab/cm^2 for MCB) were applied on the surface of 2 ml of diet dispensed in the cells of plastic trays (Bio-Ba-128, Color-Dec Italy, Capezzano Pianore, Italy). The controls consisted of Triton X-100 solution at the same concentration at the highest dose tested. One neonate larva (<24 h old) was placed in each cell and confined with a cover (Bio-Cv-16, Color-Dec Italy, Capezzano Pianore, Italy). A total of 48 larvae was tested at each concentration, and they were taken from each of the oviposition cages to preserve variability. Trays were incubated in growth chambers at 25 ± 0.3 °C, 70 ± 5% r.h., and constant dark. Mortality (larvae not showing any reaction when prodded) was assessed after 7 days.

Laboratory selection

Selection assays were performed with a population of MCB established in 2000 with larvae collected from Ebro and Badajoz, and with a population of ECB initiated in the same year with specimens from Andalucía and Badajoz. At the F_1 generation, both laboratory populations were split into two strains, one submitted to selection (R-strain) and the other maintained as a control (C-strain). Larvae of the R-strains were exposed to increased concentrations of Cry1Ab over eight generations. The concentrations of Bt toxin used for each species were established according to preliminary tests, to obtain a mortality percentage of about 80%. With each generation, a minimum of 1300 and 2500 neonate larvae of MCB and ECB, respectively, were reared over 5–7 days on a meridic diet that contained the toxin, and the surviving larvae were transferred to an untreated diet until they completed their development. Egg masses were obtained from several oviposition cages, as above, to preserve variability. Plastic trays (Bio-Ba-128, Color-Dec Italy, Capezzano Pianore, Italy) were used as before, but five and 10 neonate larvae of MCB and ECB were placed in each cell, respectively. Every four generations, the susceptibility to Cry1Ab of the neonate larvae from R- and C-strains of the two species was tested as described above.

Data analysis

For each field or laboratory population, mortality data were used to determine their susceptibility to Cry1Ab (LC_{50} with a 95% confidence interval) by probit analysis using the computer program POLO-PC (LeOra Software). The significance of changes in susceptibility was tested by the 95% confidence limits of lethal concentration ratios (LCR) at the LC_{50} (Robertson & Preisler, 1992).

Results

The susceptibility of field populations of MCB to Cry1Ab toxin during the period 1999–2002 is shown in Table 1. Changes in susceptibility within a population were determined by comparing their lethal concentrations with respect to the susceptibility of the first sampling year. No significant differences in susceptibility were obtained when the Ebro population was sampled in successive years during this period. Likewise, no significant difference was found for the susceptibility of the Badajoz population collected in 2002 with respect to its susceptibility in 2000. The Madrid population sampled in 2001 showed a reduced susceptibility when compared to 1999, but the differences were not significant in 2000 and 2002. In contrast, when the Albacete population was sampled in 2000 its susceptibility was significantly higher than in 1999. The susceptibility of a MCB strain maintained in the laboratory

Table 1 Susceptibility of field-collected larvae of *S. nonagrioides* from different Spanish maize growing areas to Cry1Ab

Population	Year	n	Slope ± SE	χ^2	d.f.	LC_{50}[a] (95% CL)	LCR (LC50)[b] (95% CL)
Madrid	1999	336	1.33 ± 0.33	7.4	12	5 (1–9)	1.00
	2000	427	1.89 ± 0.29	21.6	16	10 (5–15)	1.88 (0.57–6.17)
	2001	432	1.87 ± 0.28	2.8	7	19 (13–26)	3.69 (1.13–12.12)[c]
	2002	384	1.50 ± 0.17	31.5	14	15 (9–23)	2.84 (0.91–8.89)
Ebro	1999	429	2.38 ± 0.35	8.8	7	23 (14–31)	1.00
	2000	336	3.10 ± 0.54	1.4	5	20 (15–25)	0.89 (0.59–1.35)
	2001	432	1.95 ± 0.30	3.9	7	34 (27–42)	1.52 (1.00–2.25)
	2002	384	1.56 ± 0.21	12.5	10	11 (7–16)	0.51 (0.09–2.76)
Albacete	1999	336	1.82 ± 0.34	4.7	5	15 (10–20)	1.00
	2000	152	2.21 ± 0.34	12.0	8	9 (6–12)	0.60 (0.38–0.97)[c]
Badajoz	2000	333	3.12 ± 0.67	10.7	5	18[d]	1.00
	2002	334	2.12 ± 0.21	33.1	15	22 (16–32)	1.27 (0.76–2.12)
Laboratory	2000	304	2.77 ± 0.47	9.5	8	8 (5–11)	1.00
	2001	336	2.96 ± 0.35	9.9	12	5 (4–6)	0.70 (0.46–1.08)
	2002	384	3.15 ± 0.42	13.0	12	10 (7–12)	1.18 (0.77–1.81)

[a]Concentrations expressed in ng Cry1Ab cm^{-2}.
[b]Lethal concentration ratio (LCR) at LC_{50} with respect to the susceptibility baseline or the first sampling year on Bt-maize fields.
[c]Lethal concentrations significantly different ($P < 0.05$) if the LCR 95% confidence interval does not include 1.
[d]95% CL could not be estimated because the coefficient g was >0.5 at the 95% probability level.

remained relatively constant during the period 2000–2002 (Table 1).

The susceptibility of field populations of ECB to Cry1Ab toxin during the period 1999–2002 is shown in Table 2. The susceptibility of the Badajoz population remained at similar levels in 1999, 2000, and 2002, whereas a significant increase in susceptibility was observed when the Ebro population was sampled in 2002, compared with 2001. The susceptibility of an ECB laboratory strain remained stable for 3 years (Table 2).

Selection assays were performed with MCB and ECB laboratory strains for eight generations, and the susceptibility to Cry1Ab of the control and selected strains was tested every four generations (Table 3). The MCB R-strain was 16-fold significantly more tolerant to Cry1Ab than the unselected C-strain after four generations of selection, and the differences significantly increased to 21-fold after eight generations. Laboratory selection for four and eight generations yielded an ECB R-strain 4- and 10-fold significantly more tolerant to Cry1Ab than the C-strain, respectively.

Discussion

Resistance management strategies to ensure the long-term effectiveness of Bt plants are dependent on the development of effective resistance monitoring programs capable of early detection of resistance to allow the implementation of appropriate management decisions in a timely

Table 2 Susceptibility of field-collected larvae of *O. nubilalis* from different Spanish maize growing areas to Cry1Ab

Population	Year	n	Slope ± SE	χ^2	d.f.	LC_{50}[a] (95% CL)	LCR (LC_{50})[b] (95% CL)
Ebro	2001	400	2.96 ± 0.41	4.8	6	34 (28–41)	1.00
	2002	384	2.10 ± 0.21	21.6	13	9 (6–12)	0.25 (0.19–0.34)[c]
Badajoz	1999	959	0.92 ± 0.07	42.8	16	4 (2–6)	1.00
	2000	336	1.43 ± 0.21	19.9	13	3 (2–5)	0.79 (0.48–1.31)
	2002	384	1.98 ± 0.17	4.8	5	6 (5–7)	1.58 (1.00–2.29)
Laboratory	2000	1103	1.60 ± 0.10	42.8	21	3 (2–4)	1.00
	2001	336	1.79 ± 0.30	8.4	10	4 (3–6)	1.28 (0.80–2.04)
	2002	333	2.06 ± 0.29	13.4	12	3 (2–4)	0.87 (0.57–1.33)

[a]Concentrations expressed in ng Cry1Ab cm^{-2}.
[b]Lethal concentration ratio (LCR) at LC_{50} with respect to the susceptibility baseline or the first sampling year on Bt-maize fields.
[c]Lethal concentrations significantly different ($P < 0.05$) if the LCR 95% confidence interval does not include 1.

Table 3 Susceptibility of laboratory-selected and unselected control strains of *O. nubilalis* and *S. nonagrioides* to Cry1Ab toxin

Species	Strain (generation)[a]	n	Slope ± SE	χ^2	d.f.	LC_{50}[b] (95% CL)	LCR (LC_{50})[c] (95% CL)
S. nonagrioides							
	C(4)	288	2.49 ± 0.5	8.9	10	7 (4–10)	
	R(4)	336	1.38 ± 0.4	2.6	5	116 (73–391)	16 (6–45)*
	C(8)	284	3.07 ± 0.6	7.0	11	11 (7–14)	
	R(8)	209	3.08 ± 0.8	15.5	12	226 (127–320)	21 (12–36)*
O. nubilalis							
	C(4)	336	1.79 ± 0.3	8.4	10	4 (3–6)	
	R(4)	336	2.4 ± 0.4	9.9	12	17 (12–21)	4 (2.3–6.6)*
	C(8)	333	2.06 ± 0.3	13.4	12	3 (2–4)	
	R(8)	400	2.22 ± 0.3	31.5	19	29 (17–41)	10 (6.1–16.4)*

[a]Probit analysis was performed on unselected control (C) and selected (R) strains following the 4th and 8th generation.
[b]Concentrations expressed in ng Cry1Ab cm^{-2}.
[c]Lethal concentration ratio (LCR) at LC_{50} level of selected strain with respect to the unselected control strain within each generation.
[d]Lethal concentrations significantly different ($P < 0.05$) if the LCR 95% confidence interval does not include 1.

manner. Accordingly, we have assessed the level of resistance of Spanish field populations of MCB and ECB by regular monitoring on Bt maize during the period 1999–2002. Our results did not indicate an increase in tolerance to Cry1Ab toxin in the MCB populations from Ebro, Albacete, and Badajoz, or in the ECB populations from Ebro and Badajoz after 5 years of Bt maize cultivation in Spain. We found a small (3.7-fold), but significant, increase in tolerance in the MCB population sampled from Madrid in 2001 with respect to the same population sampled in 1999. However, a gradual trend towards higher levels of tolerance was not observed, since the susceptibility of the MCB population from Madrid fluctuated and was again not significantly different when sampled in 2002. It has been suggested that increases in tolerance when comparing historic data may reflect a loss of activity of the toxin during long-term storage (Siegfried et al., 2001). However, the susceptibility of the MCB and ECB laboratory strains remained relatively constant during the period 2000–2002, indicating that there were no changes in the stability of the toxin. Nevertheless, a significant decrease in the tolerance of the MCB population from Albacete between 2000 and 1999 and for the ECB population from Ebro between 2002 and 2000 would suggest that at least part of the year-to-year variation in susceptibility may reflect a non-genetic variation rather than genetically determined differences (Robertson et al., 1995; Marçon et al., 1999). Similar findings have been obtained for ECB field populations collected from Bt maize fields in the USA (Siegfried et al., 2001). It is therefore unlikely that the slight changes detected in susceptibility are the result of selection for resistance, and are more likely to be a result of natural variability. Coincident with the commercial release of Bt maize in Spain in 1998, González-Núñez et al. (2000) established the baseline susceptibility to Cry1Ab toxin of several geographically different MCB and ECB populations, including those from Madrid and Ebro. The data reported here correspond to a new batch of toxin that yielded a higher toxicity than that used by González-Núñez et al. (2000), when tested with the same populations (Farinós et al., 2001), and in the range of the lethal concentrations obtained with other ECB populations using Cry1Ab (Marçon et al., 1999; Siegfried et al., 2001). Thus, to avoid drawing erroneous conclusions we decided to restrict our comparisons to those cases where the same toxin batch was used.

Laboratory selection assays with ECB populations from different geographical areas in the USA and Europe have shown that these populations can respond very rapidly (within 10 generations) to intense selection pressure with Bt toxins (Bolin et al., 1999; Chaufaux et al., 2001) or commercial formulations of Bt (Huang et al., 1997). The relevance of laboratory selection assays to forecast the development of insect resistance in the field has been questioned, because the selection pressure is lower than in the field, where the larvae are exposed to high concentrations of Bt toxin throughout the season (Chaufaux et al., 2001). In Spain, where only Compa CB is cultivated, larvae of the second and third generation are exposed to sublethal doses of the toxin and therefore laboratory selection might be more relevant than in other situations where a high expression of Bt toxin is maintained throughout the maize season. Selection for resistance in ECB and MCB populations collected from different Spanish geographical areas has resulted in laboratory strains with increased levels of resistance to Cry1Ab. The level of resistance that we achieved

with the ECB population after eight generations of selection was 10-fold, somewhat smaller than the 14–32 fold resistance to Cry1Ab obtained by Chaufaux et al. (2001) with three ECB populations from Italy, Nebraska, and a combination of France and Switzerland. The smaller response to selection in the Spanish population may be caused by differences in the genetic background of the populations or in the toxin/formulation used for the selection. Nevertheless, these studies suggest that the potential to develop low to moderate levels of tolerance may be relatively common among widely distributed ECB populations. The level of tolerance obtained for MCB after eight generations of selection was also moderate (21-fold), demonstrating that loss of susceptibility to Bt toxins can also develop in MCB populations. The relevance of the novel results obtained in this study for MCB are due to the fact that there are no previous data on the response to selection pressure to Bt toxins in this species.

Although differences in sensitivity among populations in a laboratory assay to Bt may be significant, those differences may not necessarily translate to a reduced field efficacy of Bt plants. In fact, none of the field-collected or laboratory-selected MCB and ECB larvae were able to survive on Bt maize seedlings (data not shown), and there were no field records of corn borers with high levels of resistance which were capable of surviving on Bt maize plants expressing the toxin. The resistance to Dipel of the laboratory-selected ECB strain obtained by Huang et al. (1997) appears to be inherited as an incompletely dominant autosomal gene (Huang et al., 1999). However, the practical importance of this genetic dominance will depend on whether these insects can survive on Bt maize. Huang et al. (2002) has reported that Dipel-resistant ECB larvae were not able to survive to adulthood on whorl-stage MON810, Bt11, Bt176 and DBT418 event maize plants. Using a sibling mating method (F_2 screen) for estimating resistance allelic frequencies, Andow et al. (1998, 2000) found no major resistance alleles conferring resistance to Bt maize among 279 isofemale lines screened from two ECB populations from Minnesota and Iowa, whereas partial resistance was often found (the frequency of partial resistance alleles was 3.9×10^{-3} in the Iowa population). An additional F_2 screen has confirmed such a high frequency for partial resistance alleles in an ECB population from southern France (Chaufaux et al., 2001). As a consequence, it appears that alleles for partial resistance may be reasonably common in natural ECB populations, but alleles for complete resistance to the toxin may be rare.

Current resistance management strategies for Bt crops are mainly based on the implementation of the 'high-dose/refuge' strategy, which involves the use of plants expressing the toxin at a level which is high enough to kill heterozygous resistant insects, and the provision of refuges of non-transgenic plants on which sensitive insects could multiply, allowing the random mating of sensitive and resistant insects (Alstad & Andow, 1995). However, agro-ecological factors should be considered for the adaptation of resistant management strategies to different geographical areas. Remarkably, several features occurring in Spain and other Mediterranean countries may affect the success of the 'high-dose/refuge' strategy: (i) Bt maize varieties based on the 176 event, such as Compa CB grown in Spain, are more likely to allow the survival of a second or third generation of heterozygous resistant larvae (Onstad & Gould, 1998; Walker et al., 2000); (ii) it has been shown that MCB females mate before they move for oviposition (López et al., 1999), so that females emerging from refuge would rarely mate with potential resistant moths emerging from Bt maize fields and vice versa; and (iii) ECB mobility is also reduced before oviposition in irrigated maize fields (Hunt et al., 2001), which corresponds to the agronomic practices of most maize growing areas in Spain. These issues highlight the need to adapt resistance management strategies to local areas, and to strengthen the usefulness of systematic field monitoring for early detection of resistance in Spain, even though the selection pressure for adaptation has likely been minimal, given that only 5% of the maize is planted to Bt cultivars.

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