Managing the evolution of *Bacillus thuringiensis* resistance in natural populations of the European corn borer, *Ostrinia nubilalis*: host plant, host race and pherotype of adult males at aggregation sites

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The European corn borer (ECB) consists of at least two, genetically differentiated host races: one feeding on maize, the other feeding on mugwort and hop. It is unclear to what extent individuals feeding on these, or other host plants, contribute to natural ECB populations. The mechanisms underlying the genetic differentiation between both races are not well understood; they may include sexual attraction via different pheromone blends (E or Z) and differences in the location of mating sites. We caught adult males with traps baited with the E or the Z blend at hop, maize, and 'mixed' sites. We determined their probable host race by allozyme-based genetic assignment, and the photosynthetic type of their host plant by stable carbon isotope analysis. Most individuals caught in Z traps had emerged from a C₄-type plant and belonged to the maize race, whereas most individuals caught in E traps had emerged from C₃-type plants and were but weakly differentiated from the hop–mugwort race, suggesting a strong, though not absolute, correspondence between host plant, host race and pherotype. We also found that although spatial segregation may contribute to genetic isolation between host races, moths of both host races may be present at a given location. Regarding the management of *Bacillus thuringiensis* (Bt) maize, our results indicate that, at least at the present study sites, it is unlikely that any wild or cultivated C₃-type plant species could be a source of susceptible individuals that would mate randomly with Bt-resistant Z-C₄ moths emerging from Bt-maize fields.

Keywords: pheromone; transgenic insecticides maize; stable carbon isotopes; *Bacillus thuringiensis*; pest resistance management; genetic assignment

1. INTRODUCTION

Transgenic maize varieties producing insecticidal *Bacillus thuringiensis* (Bt) toxins ('Bt-maize') have been developed to control infestations of the European corn borer (ECB) *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), a polyphagous pest feeding on maize and on more than 200 other wild and cultivated host species (Hodgson 1928). Owing to fears that alleles conferring resistance to Bt toxins might be rapidly selected in ECB populations (Gould 1998), the so-called 'high dose–refuge' strategy (Alstad & Andow 1995) has been made mandatory in the USA and is being considered for Bt-maize management in Europe. Resistance is assumed to be monogenic and recessive, and the 'high dose' of toxin to be sufficient to kill both heterozygotes and susceptible homozygotes. The 'refuge' can be any host plant species, provided it is Bt-toxin-free (and therefore does not select for resistance) and provides sufficient

numbers of susceptible ECB adults. These susceptible moths must mate randomly with moths emerging from Bt-maize fields, to dilute any Bt resistance alleles selected. The costs associated with the infestation of 'sacrificial' non-Bt-maize refuges with sufficiently large ECB populations has led to a search for alternative plants that could play such role: wild hosts spontaneously growing in the vicinity of maize fields, or cultivated plants unharmed by the presence of ECB. At least two such species have been identified in France (Bourguet *et al.* 2000a; Martel *et al.* 2003): mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.). However, allozyme-based studies have shown that ECB populations collected as larvae on mugwort and hop are genetically differentiated from sympatric larval populations collected on maize (Bourguet *et al.* 2000a; Martel *et al.* 2003), and that this differentiation in genetic structure also exists at the adult stage (Thomas *et al.* 2003). It is therefore unlikely that moths emerging from hop or mugwort will mate randomly with those from maize fields. Thus, neither of these plants can be considered to be an efficient refuge.

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Figure 1. Geographical locations of the sampling sites: (1) Brugières-maison, (2) Brugières-pont, (3) Pradal-Pierrelatte, (4) Saint-Marcel, (5) Lambrois, (6) X-Files, (7) Dupré and (8) Pradal-maison. Sites 1–3 were located in the vicinity of maize fields, sites 4 and 5 were located close to clumps of wild hop, and sites 6–8 were located at the border between a maize field and wild hop clumps.

The genetic divergence between ‘maize’ populations and ‘hop–mugwort’ populations may be due to differences in sexual pheromones: indeed, ECB males collected from maize are attracted to the so-called Z pheromone blend, which is produced by the ECB females collected on maize, whereas males collected from hop or mugwort are attracted to the E blend, which is produced by the females collected from hop or mugwort (Thomas *et al.* 2003; Pélozuelo *et al.* 2004). Both field (Pélozuelo *et al.* 2004) and laboratory studies (Bethenod *et al.* 2004) have provided evidence that hybrid mating between ‘maize’ and ‘hop–mugwort’ moths is rare and/or that the offspring of such matings are generally not fertile. This suggests that ECB consists of at least two host races, one with the Z phenotype and developing on maize (which was introduced into Europe *ca.* 500 years ago), and another, possibly the ancestral race, with the E phenotype, feeding on mugwort, hop and possibly other native European plants.

However, the correspondence between host plant, host race and phenotype may not be as strict as it would first appear. Indeed, as ECB has more than 200 documented host plants (Hodgson 1928), some Z and/or E moths in the field could emerge from hosts other than maize, mugwort and hop. Similarly, some Z and/or E moths may not share the genetic characteristics of the maize and hop–mugwort races, respectively. Finally, factors other than the pheromone-based mating recognition system, such as differences in spatial location, may contribute to assortative mating, resulting in genetic isolation between host races. Indeed, adult ECBs usually mate in grass patches, called aggregation sites, where they also tend to stay during the day. There is no evidence to date that males attracted by and females producing the E and Z pheromones are found together at the same aggregation sites. A separation of phenotypes might occur, either because insects tend to aggregate close to patches of their host plant or because of long-range pheromone attraction, resulting in the formation of separate Z and E aggregations.

In this study we aimed (i) to search for evidence in the field for the existence of Z or E moths not sharing the genetic characteristics of the maize and hop–mugwort races, respectively, (ii) to search for evidence in the field that plants other than maize and hop may be significant sources of Z and E individuals, respectively (mugwort is not infested in our study area: Martel *et al.* 2003), and (iii) to evaluate the effect of the immediate vicinity of maize or hop

plants on the spatial distribution of individuals from different host races, phenotypes or host plants. We did this by placing pairs of pheromone traps—one baited with the E blend and the other with the Z blend—at three kinds of site: maize, hop and mixed. Trapped individuals were examined genetically, by allozyme-based methods, to determine whether they belonged to one or the other of the two known host races). We also identified the host plant group of origin of the insects trapped by determining their stable carbon isotope content.

2. MATERIAL AND METHODS

(a) *Sampling sites and pheromone trapping*

Eight sampling sites were chosen in the surroundings of Bourg-Saint-Andéol (Ardèche, France; figure 1): three maize field borders, two borders of large wild hop bushes and three ‘mixed’ sites in the immediate vicinity of both maize and wild hop. All sites were within a distance of 10 km of each other, making genetic isolation by distance between ECB populations unlikely (Bourguet *et al.* 2000*a,b*).

A pair of traps (Delta traps, Biosystèmes, France; or Scentry traps, Gemplers, USA) was left for several sets of several days at each site between 1 June and 31 August 2002. The two traps in each pair were *ca.* 50 m apart; one trap was baited with a lure releasing the E pheromone blend, and the other with a lure releasing the Z blend. The E blend contained 97% *E*- Δ -11-tetradecenylacetate and 3% *Z*- Δ -11-tetradecenylacetate; the Z blend contained the same two isomers but with the proportions inverted (3/97). These proportions correspond to those usually released by females of the E and Z phenotype, respectively (Klun *et al.* 1973; Kochansky *et al.* 1975; Cardé *et al.* 1978). Lures were purchased from Biosystèmes, France. The locations of the E and Z traps at a given site were regularly interchanged, to prevent within-site effects of trap location on the final proportion of catches in the two traps. Trapped males were collected every 1–3 days, immediately frozen (-20°C) and stored frozen until further analysis.

Diapausing larvae were also collected in the autumn of 2002 at a subset of the sites at which the pheromone traps had been in operation: two samples were taken from maize stalks (in Brugières-maison, $n = 43$, and in X-Files, $n = 44$) and two from wild hop stalks (in Lambrois, $n = 44$, and in X-Files, $n = 30$).

(b) *Stable carbon isotope analysis*

The wings and legs of each moth were cut from the rest of the body and freeze-dried. They were then folded, without prior grinding, in stainless tin cups and sent for stable carbon isotope

analysis to the Scottish Crop Research Institute (Dundee, Scotland). Results are expressed in conventional 'δ' units relative to the Pee Dee Belemnite standard as: $\delta^{13}\text{C}(\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000$, where R is the $^{13}\text{C}/^{12}\text{C}$ atoms ratio. Animal tissues closely mirror the $\delta^{13}\text{C}$ value of their food (De Niro & Epstein 1978). As the $\delta^{13}\text{C}$ values of plants with C_3 - and C_4 -type photosynthesis differ, the animals consuming these two types of plants may be distinguished according to the $\delta^{13}\text{C}$ value of their tissues (e.g. Gould *et al.* 2002). As ECB larvae typically go through their entire development process on a single plant, the wing $\delta^{13}\text{C}$ of adults can be used to determine the photosynthetic type of this plant. Ponsard *et al.* (2004) showed that, although ECB adults have functional mouth parts and feed on the nectar of various plants (Beck 1987), adult feeding has too small an effect on ECB wing $\delta^{13}\text{C}$ to obscure the isotopic signal corresponding to the host plant of the larva.

(c) *Allozymes*

The sex of each moth or larva was recorded and its head removed and kept for further analysis. The body was then homogenized in 150 µl of Tris-EDTA (pH 6.8). The homogenates were subjected to horizontal starch gel electrophoresis in Tris-borate-EDTA (pH 8.6) buffer systems (Pasteur *et al.* 1987). We investigated six polymorphic enzymes allowing unequivocal genetic interpretation, as previously described (Bourguet *et al.* 2000b). These enzyme systems were: phosphoglucumutase (PGM, EC 8.4.2.2), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), hydroxybutyrate dehydrogenase (HBDH, EC 1.1.1.30), glucose-phosphate isomerase (GPI, EC 5.3.1.9), aspartate-aminotransferase (AAT, EC 2.6.1.1) and triose-phosphate isomerase (TPI, EC 5.3.1.1).

(d) *Data analysis*

Allelic frequencies were estimated at each locus for each population (data available upon request from D.B.). We used GENEPOP 3.1d software (Raymond & Rousset 1995) to check that there was no deviation from Hardy-Weinberg equilibrium or linkage disequilibrium between pairs of loci. This program was also used to analyse the genetic structure of populations by calculating the θ estimator of F_{st} as described by Weir & Cockerham (1984) and by testing for genetic differentiation using Fisher's exact tests.

We assigned individuals or groups of individuals to a given population on the basis of allele frequencies. We used a multinomial distribution to compute the likelihood that the to-be-assigned individuals or groups of individuals belong to each candidate population of origin (Paetkau *et al.* 1995). A Monte Carlo resampling algorithm (Paetkau *et al.* 2003) was then used to compute the probability of each of these individuals or groups having originated from each candidate population of origin. These analyses were performed with GENECLASS2 software (Piry *et al.* 2004).

The distribution of stable carbon isotope values was tested for unimodality by means of Hartigan's dip test (1985) performed with the Diptest package of R software (www.r-project.org).

3. RESULTS

At maize and mixed sites, more than 94% of the males were captured in traps baited with Z pheromone lures. By contrast, at the only hop site where a substantial number of individuals were caught, most (*ca.* 80%) of them were captured in traps baited with the E pheromone blend (table 1). All males captured in E pheromone traps except three with damaged wings ($n = 2, 1, 8, 39$ and 1 for Brugières-maison, Lambrois, Pradal-maison, St Marcel and X-Files,

Table 1. Number of moths captured at each site in traps baited with E or Z pheromone lures.

habitat	site	pheromone lure	
		E	Z
maize	Brugières-maison	2	49
	Brugières-pont	0	63
	Pradal-Pierrelatte	0	47
	total	2 (1.2%)	159 (98.8%)
hop	Lambrois	1	8
	Saint-Marcel	40	6
	total	41 (74.5%)	14 (25.5%)
maize + hop	X-Files	1	25
	Dupré	0	72
	Pradal-maison	10	109
	total	11 (5.1%)	206 (94.9%)

respectively; n total = 51) and up to 54 males per site captured in the Z pheromone traps ($n = 39, 19, 20, 8, 54, 20, 6$ and 20 for Brugières-maison, Brugières-pont, Dupré, Lambrois, Pradal-maison, Pradal-Pierrelatte, St Marcel and X-Files, respectively; n total = 186) were analysed to determine their stable carbon isotope ratio and their allozyme profiles for the six loci considered.

The distribution of $\delta^{13}\text{C}$ values is given in figure 2: it is clearly bimodal (the null hypothesis of unimodality was rejected according to Hartigan's (1985) dip statistic = 0.041, $p < 0.05$, $n = 237$), with one group of 50 individuals with values ranging from -30.0 to -22.0‰ (typical C_3 values) grouped around a single mode (Hartigan's dip statistic = 0.045, $p > 0.50$, $n = 50$), and another group of 187 individuals with values ranging from -20.2 to -10.7‰ (typical C_4 values), also grouped around a single mode (Hartigan's dip statistic = 0.020, $p > 0.50$, $n = 187$). Table 2 shows the total number of individuals with a C_3 - or a C_4 -type $\delta^{13}\text{C}$ value caught in E or in Z traps. The pheromone bait in the trap (E or Z) and the host plant type of origin (C_3 or C_4) of the trapped individuals were highly significantly linked to each other (Fisher's exact test: $p < 10^{-5}$): as expected, most individuals (86.3%) caught in E traps had a C_3 -like $\delta^{13}\text{C}$ value, whereas most individuals (96.8%) caught in Z traps had a C_4 -like $\delta^{13}\text{C}$ value. However, 13.7% of the individuals caught in E traps had developed on a C_4 plant and 3.2% of the individuals caught in Z traps had developed on a C_3 plant, suggesting that the correspondence between phenotype and host-plant type, while strong, is not absolute. The four categories of individuals will be referred to hereafter as E- C_3 , Z- C_4 , E- C_4 and Z- C_3 , respectively.

As expected, there was no evidence for genetic differentiation between the two populations of diapausing larvae collected on hop, or between the two populations of diapausing larvae collected on maize (details not shown). Diapausing larvae were therefore grouped into a single population per host plant. No genetic differentiation was found between the E- C_3 or between the Z- C_4 moth populations trapped at the different sites (details not shown), so all E- C_3 individuals were grouped together, and all Z- C_4 individuals were grouped together, regardless of site. Diapausing larvae collected on hop displayed significant differentiation from those collected on maize (table 3; θ over all loci = 0.0625, $p < 10^{-5}$) and E- C_3 adults differed

Figure 2. The $\delta^{13}\text{C}$ values of males captured, for all traps. The null hypothesis of unimodality was rejected ($p < 0.05$) for the overall distribution of $\delta^{13}\text{C}$ values, but it was not rejected ($p > 0.5$) when the sets of values less than -22.0‰ and greater than -20.2‰ were considered separately (Hartigan's Dip test, see § 2d).

Table 2. Proportion (%) of males from C_3 and C_4 host plants captured in E and Z traps.

pherotype	<i>n</i>	host plant (%)	
		C_3	C_4
E	51	86.3	13.7
Z	186	3.2	96.8

significantly from Z- C_4 adults ($\hat{\theta}$ over all loci = 0.0943, $p < 10^{-5}$). Not all allozyme loci made equal contributions to the genetic differentiation observed: in diapausing larvae, the difference was mostly due to *Gpi* and *Mpi*, whereas *Tpi* allele frequencies were similar (table 3). By contrast, E- C_3 and Z- C_4 populations displayed significant differences in allelic frequencies at the *Tpi* and *Gpi* loci, but not at the *Mpi* locus (table 3). Furthermore, Z- C_4 adult males did not display any significant genetic differentiation from diapausing larvae collected on maize ($\hat{\theta}$ over all loci = -0.0006 , $p = 0.440$; table 3). More surprisingly, a significant genetic differentiation was observed between E- C_3 moths and larvae diapausing on hop ($\hat{\theta}$ over all loci = 0.0263, $p = 0.005$; table 3). *Tpi* and *Mpi* were the loci that made the largest contribution to this differentiation (table 3). This differentiation was not due to the E- C_3 moth sample consisting exclusively of males whereas the diapausing larvae sample consisted of both males and females. Indeed, as in previous studies (Bourguet *et al.* 2000a,b; Martel *et al.* 2003), male and female diapausing larvae displayed no significant genetic differentiation (details not shown).

Genetic assignment showed that three Z- C_3 individuals were more likely to belong to the E- C_3 than to the Z- C_4 population whereas the other three Z- C_3 individuals were more likely to belong to the Z- C_4 than to the E- C_3 population (table 4). Five out of seven E- C_4 individuals were found to be more likely to belong to the Z- C_4 population than to the E- C_3 population (table 4). However, only one Z- C_3 individual (95 SM) could be assigned unambiguously

to one of the two main categories (table 4). Indeed, this individual could be safely excluded from the Z- C_4 population ($p = 0.003$), but not from the E- C_3 population ($p = 0.102$). For all other individuals, neither the Z- C_4 nor the E- C_3 group could be rejected as the population of origin (all p values greater than 0.10; see table 4). As a group, Z- C_3 moths were assigned equally frequently to the E- C_3 population and Z- C_4 populations (table 4). By contrast, E- C_4 moths were found to be more likely to belong to the Z- C_4 population than to the E- C_3 population.

4. DISCUSSION

The photosynthetic type of the larval host plant of 237 adult ECB males trapped in the field was unambiguously determined on the basis of their wing $\delta^{13}\text{C}$ values (figure 2). The $\delta^{13}\text{C}$ distribution was clearly bimodal, with no overlap, and with values typical of ECB adults emerging from mugwort or maize (Ponsard *et al.* 2004) and other consumers of C_3 or C_4 plants (De Niro & Epstein 1978; Gould *et al.* 2002). We therefore considered the first group to have emerged from a C_3 plant (e.g. hop), and the second to have emerged from a C_4 plant (probably maize). The 224 documented ECB host plants include 19 species with C_4 photosynthesis (Ponsard *et al.* 2004): two tropical species that do not grow in Europe, 14 wild or ornamental species planted over only small areas, and three cultivated cereals—millet, sorghum and maize. Only maize and sorghum are cultivated to any significant extent in France, with maize largely predominating (more than 3×10^6 ha versus *ca.* 5×10^4 ha, respectively: <http://www.agreste.agriculture.gouv.fr>). Sorghum is cultivated in the vicinity of our study sites, but in marginal amounts compared with maize surfaces (A.B., D.B., L.P. and S.P., personal observation). In addition, sorghum usually displays lower levels of infestation per unit area than maize, as shown by observations in the former Soviet Union (Dyatlova & Frolov 1999), and both southwestern and southeastern France (L. Leniaud, L.P., D.B. and S.P., unpublished data). Hence, individuals with a C_4 -type $\delta^{13}\text{C}$ value are much more likely to have emerged from maize than from any

Table 3. Genetic differentiation between males emerged from C₃ host plants and captured in E pheromone traps (E-C₃ males), males emerged from C₄ host plants and captured in Z pheromone traps (Z-C₄ males), diapausing larvae collected from hop stalks (hop larvae) and diapausing larvae collected from maize stalks (maize larvae). (Number of individuals equals 43, 180, 74 and 87 for E-C₃ males, Z-C₄ males, hop larvae and maize larvae, respectively.)^a

comparison	loci						
	<i>Tpi</i>	<i>Gpi</i>	<i>Hbdlh</i>	<i>Aat</i>	<i>Mpi</i>	<i>Pgm</i>	all
E-C ₃ males versus Z-C ₄ males	$\hat{\theta}$ 0.1767 p < 10 ⁻⁵	0.1374 < 10 ⁻⁵	-0.0079 1.000	0.0424 0.0293	0.0203 0.2116	0.0284 0.048	0.0943 < 10 ⁻⁵
maize larvae versus hop larvae	$\hat{\theta}$ -0.0055 p 0.390	0.0959 < 10 ⁻⁵	-0.0009 1.000	0.0586 < 10 ⁻⁴	0.0710 < 10 ⁻⁵	0.0230 0.008	0.0625 < 10 ⁻⁵
E-C ₃ males versus hop larvae	$\hat{\theta}$ 0.0946 p 0.008	-0.0014 0.439	— ^a — ^a	0.0063 0.255	0.0299 0.005	-0.0078 0.859	0.0263 0.005
males Z-C ₄ versus maize larvae	$\hat{\theta}$ -0.0067 p 0.651	-0.0032 0.547	-0.0033 0.553	0.0038 0.052	0.0029 0.452	0.0006 0.516	-0.0006 0.440
E-C ₃ males versus maize larvae	$\hat{\theta}$ 0.1441 p < 10 ⁻³	0.1489 < 10 ⁻⁵	-0.0071 1.000	0.0158 0.036	-0.0009 0.223	0.0066 0.111	0.0860 < 10 ⁻⁵
Z-C ₄ males versus hop larvae	$\hat{\theta}$ -0.0022 p 0.130	0.0854 < 10 ⁻⁵	-0.0028 1.000	0.1083 < 10 ⁻⁵	0.0832 < 10 ⁻⁵	0.0504 < 10 ⁻³	0.0741 < 10 ⁻⁵

^a The absence of polymorphism in E-C₃ males and in diapausing larvae collected on hop precluded the calculation for these two groups of individuals.

other C₄ ECB host plant. Conversely, individuals with a C₃-type $\delta^{13}\text{C}$ may have emerged from hop or from any of the more than 200 other C₃ ECB host plants, a large number of which are temperate cultivated or common wild species that are likely to be found in the vicinity of our study sites (Ponsard *et al.* 2004).

Individuals emerging from both types of host plant were trapped at the same site, as shown by the results for hop and mixed sites. Therefore, although spatial segregation may contribute to genetic isolation between host races, C₃ and C₄ moths appear to be truly sympatric, even on a fine spatial scale. The pheromone lures may have attracted a few individuals that were not present in the immediate vicinity. The exact range of these traps is not known. However, long-range attraction is unlikely to be entirely responsible for what would be a 'spurious' co-occurrence of both phenotypes, as the E type was predominant at the hop site Saint-Marcel even though Z individuals from maize were present in much larger numbers in this area.

The results of our pheromone trapping experiments show that the apparent male phenotype (i.e. the pheromone blend with which the trap was baited) was predominantly Z type at maize (98.8% individuals) and mixed sites (94.9%), and predominantly E type (87.0%) at Saint-Marcel, the only hop site where a substantial number of individuals was caught (or 74.5% when taking into account both hop sites: table 1). These results suggest that the predominant phenotype of ECB males is strongly associated with the prevalence of their host plant in the surroundings. The phenotype of most individuals also matches their host plant type, as 96.8% of individuals trapped in a Z trap had $\delta^{13}\text{C}$ values typical of C₄ plants whereas 86.3% of individuals trapped in an E trap had $\delta^{13}\text{C}$ values typical of C₃ plants. However, this correspondence between characters was not absolute, as we trapped a few C₄ and C₃ males in E and Z traps, respectively (table 2).

We did not expect to find seven E-C₄ and six Z-C₃ individuals, given that Pélozuelo *et al.* (2004) found that all the larvae they collected from maize had a Z phenotype (except one hybrid among 176 individuals analysed) and all the larvae collected from hop or mugwort had an E phenotype (bar one hybrid among 75 individuals on hop, and 1 hybrid among 125 individuals on mugwort). There may be three non-mutually exclusive explanations for our results. The first is that Z-C₃ and E-C₄ males are in reality E-C₃ and Z-C₄ individuals, respectively. Indeed, the type of pheromone bait of the trap in which an individual was caught (E or Z blend) may not be a totally reliable indicator of that individual's phenotype: some males may have been caught in a given trap 'by chance', not because they were attracted to that particular phenotype blend. The fact that other insects, including ECB females, occasionally get caught in pheromone traps (A.B., personal observation) indicates that this is possible. Furthermore, most E-C₄ males were better assigned genetically to the Z-C₄ population than to the E-C₃ population. Finally, Glover *et al.* (1990) showed that *ca.* 3% of the E-race males are attracted to the Z pheromone blend whereas no Z-race males respond to the E pheromone blend. These proportions do not differ significantly from the proportion of 'mismatches' in our trapping data (Fisher's exact test: $p = 1$). The second possible explanation for the presence of E-C₄ and Z-C₃ individuals in our dataset is that they

Table 4. Genetic assignment of Z-C₃ and E-C₄ individuals to E-C₃ and Z-C₄ populations.

(The criterion L corresponds to the likelihood that an individual or a group of individuals (here Z-C₃ and E-C₄) belongs to a reference population (E-C₃ and Z-C₄ populations). The p values correspond to the type-I error associated with the exclusion of each Z-C₃ or E-C₄ individual from E-C₃ and Z-C₄ populations; n : number of allozyme loci used for genetic assignment.)

group	individual	n	population			
			E-C ₃		Z-C ₄	
			$-\log_{10}(L)$	p	$-\log(L)$	p
Z-C ₃	105 L	5	1.558	0.962	0.549	0.954
	95 SM	6	3.909	0.102	5.574	0.003
	96 SM	4	1.051	0.961	1.196	0.661
	383 XF	6	1.540	0.686	1.805	0.458
	189 PM	4	4.069	0.738	3.793	0.469
	193 PM	5	1.720	0.996	0.928	0.972
	all	6	6.631	—	6.629	—
E-C ₄	106 SM	5	1.360	0.994	1.072	0.973
	302 BM	5	1.558	0.962	0.549	0.972
	352 BM	5	1.316	0.985	1.410	0.646
	74 PM	4	1.361	0.801	0.471	0.944
	22 PM	6	1.360	0.725	1.074	0.772
	57 PM	6	2.249	0.465	2.072	0.356
	58 PM	6	2.178	0.489	2.761	0.157
	all	6	5.526	—	3.553	—

may be hybrids between the E and Z races that are attracted to both pheromone blends. If we assume that all these individuals are hybrids between the hop and maize races and that all C₃ and C₄ individuals emerged from hop and maize, respectively, then the frequency of these individuals in our sample is significantly higher than the proportions of hybrids found by Pélozuelo *et al.* (2004) on hop (Fisher's exact test, $p = 0.016$) but not significantly different from the proportions of hybrids found by Pélozuelo *et al.* (2004) in maize ($p = 0.067$). Finally, we cannot rule out the possibility that the individuals we sampled were actually Z-C₃ and E-C₄ individuals.

The Z-C₄ population displayed genetic differentiation from the population of larvae diapausing on wild hop, but not from the population diapausing on maize (table 3), confirming that most, if not all, Z-C₄ males belong to the maize race described in previous studies (Bourguet *et al.* 2000a; Martel *et al.* 2003; Thomas *et al.* 2003). The E-C₃ population displayed genetic differentiation from the larvae collected on maize. More surprisingly, this population also showed weak but significant differentiation from the population of larvae diapausing on wild hop (table 3). This suggests that the E-C₃ population is not a random sample of the hop–mugwort race previously described (Bourguet *et al.* 2000a; Martel *et al.* 2003; Thomas *et al.* 2003). Two non-exclusive explanations may account for this observation: (i) the E-C₃ population may be divided into several host races, the hop–mugwort host race being one such race, or (ii) the E-C₃ moths may belong exclusively to the hop–mugwort race but, as previously shown for the apple maggot fly *Rhagoletis pomonella* (Feder *et al.* 1993, 1997), selective pressure during the larval and/or adult stages may result in significant differences in allozyme frequencies between these two stages. The fact that the loci most responsible for the overall differentiation between E-C₃ adults and larvae collected

on hop (*Tpi* and *Mpi*) were also found to vary between the diapausing larvae and adult populations sampled from mugwort in the study of Thomas *et al.* (2003) supports explanation (ii).

In terms of *Bt* resistance management, it was already clear that gene flow is likely to be limited between the maize and hop–mugwort races. Indeed, larvae collected from hop and mugwort show no genetic differentiation within their own populations, but significant differentiation from those collected from maize (Bourguet *et al.* 2000b; Martel *et al.* 2003). This cannot be accounted for solely by repeated post-oviposition selection at each generation, as hybrid mating between individuals from both races is rare (Bethenod *et al.*, 2004). Thus, the hop–mugwort race cannot be regarded as a potentially efficient reservoir of *Bt*-susceptibility alleles. Although nearly all ECB larvae Pélozuelo *et al.* (2004) sampled from hop belonged to the E phenotype, suggesting that individuals emerging from this host plant rarely belong to the Z phenotype, Z females may occasionally lay eggs on hop at sites where this host plant is surrounded by large, heavily infested maize fields. Other C₃ host plants may also play such a role. However, our results suggest that this is not the case, as (i) despite the presence of hop and some other wild and cultivated C₃ host plants in the study area (e.g. *Xanthium* sp., *Polygonum persicaria* L., *Helianthus annuus* L.: L.P. and S.P., personal observation), the vast majority of Z individuals, regardless of trapping site, emerged from a C₄ plant and, most importantly, (ii) some of the Z-C₃ individuals we found were more likely to belong to the E-C₃ than to the Z-C₄ population on the basis of genetic assignment. These Z-C₃ individuals were probably E-C₃ individuals that flew into the 'wrong' pheromone traps. Overall, our results indicate that, at least for the sites studied here, neither hop nor any other C₃-type plant species are likely to contribute strongly to delaying the evolution of resistance in the ECB maize

race by providing susceptible individuals able to mate randomly with resistant Z-C₄ moths emerging from Bt-maize fields.

The authors thank B. Frérot for helpful advice, and M. and Ms Pélozuelo for their hospitality during the fieldwork. The authors appreciated the thorough comments and suggestions of three anonymous referees, which helped in improving the manuscript. This work was supported by the Action Incitative 'Impact des Biotechnologies dans les agro-écosystèmes' of the Centre national de la recherche scientifique (CNRS).

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