Managing resistance to Bt crops in a genetically variable insect herbivore, *Ostrinia nubilalis*

Megan E. O'Rourke,[1,4] Thomas W. Sappington,[2] and Shelby J. Fleischer[3]

[1]*Department of Ecology and Evolutionary Biology, Cornell University, Corson Hall, E149, Ithaca, New York 14853 USA*
[2]*USDA Agricultural Research Service, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, Iowa State University, Ames, Iowa 50011 USA*
[3]*501 ASI Building, Department of Entomology, Pennsylvania State University, University Park, Pennsylvania 16802 USA*

Abstract. To slow the resistance evolution of the European corn borer (ECB) to Cry proteins expressed in transgenic *Bacillus thuringensis* (Bt) corn, the United States Environmental Protection Agency (EPA) has adopted an insect resistance management (IRM) plan that relies on a "high dose/refuge" strategy. However, this IRM plan does not consider possible ecological differences between the two ECB pheromone races (*E* and *Z*). Using carbon isotope analysis, we found that unstructured (non-corn) refuges contribute more to *E* race (18%) than to *Z* race (4%) populations of ECB in upstate New York (USA). Furthermore, feeding on non-corn hosts is associated with decreased body mass and reduced fecundity. We also show that the geographic range of *E*-race ECB is restricted within the range of the *Z* race and that *E*-race ECB are increasingly dominant in regions with increasing non-corn habitat. While the proportion of *E*-race ECB developing in unstructured refuges is higher than previously assumed, low rates of unstructured refuge use by the *Z* race, evidence for reduced fecundity when reared on non-corn hosts, and complete sympatry within the *E* race range all argue against a relaxation of current IRM refuge standards in corn based on alternative-host use. We also discuss implications of this research for integrated pest management in vegetables and IRM in Bt cotton.

Key words: Bacillus thuringensis; Bt corn; Bt cotton; European corn borer, ECB; genetically modified organism; high-dose/refuge strategy; insect-resistance management, IRM; integrated pest management, IPM; Ostrinia nubilalis; pheromone races; stable carbon isotopes.

Introduction

Insect races within a single species may have very different ecologies despite being morphologically indistinguishable (Ferrari et al. 2006, Bickford et al. 2007). This can have important implications for managing agricultural pest species that are composed of multiple races. The European corn borer (ECB; *Ostrinia nubilalis*; see Plate 1) is a classic example of an economically important agricultural pest for which management strategies and regulatory policies have largely ignored the potential differences between known host races.

Genetically modified corn (*Zea mays*) varieties that produce *Bacillus thuringensis* (Bt)-derived protein toxins in vivo have proven very effective in controlling ECB feeding damage (EPA 2001) and are planted on nearly 20% of the world's corn acreage, and 50% of US corn acreage (James 2007, USDA 2008*a*). To maintain the efficacy of transgenic Bt corn, and *B. thuringensis* insecticide sprays used by organic growers against ECB, an insect-resistance management (IRM) plan has been adopted by the United States Environmental

Protection Agency (EPA) to slow the evolution of resistance in ECB to Bt products (EPA 2001, Andow 2002). The key component of the IRM plan is the "high-dose/refuge strategy." Models and data indicate that a high-dose of insecticide that is capable of killing any heterozygous resistant insects, combined with a non-Bt refuge that will produce homozygous susceptible insects, can significantly slow the evolution of resistance to Bt (Gould 2000, Andow 2002, Qiao et al. 2008). The expectation is that homozygous susceptible insects that develop in the refuge will mate with the rare homozygous resistant individual that survives in the Bt corn, and that the resultant heterozygous offspring will be killed by the high-dose of Bt toxin in transgenic corn. As of 2006, the EPA requires farmers using Bt corn in non-cotton-growing regions to plant a 20% non-Bt corn refuge within 0.8 km of Bt corn as a "nursery" for Bt-susceptible ECB (EPA 2006*b*).

For polyphagous insects, unstructured refuges (including non-corn crops and non-cropped areas) potentially could substitute for the structured refuges (non-Bt corn) mandated by EPA (Bontemps et al. 2004, Andow and Zwahlen 2006, EPA 2008). In the case of ECB, there is uncertainty concerning its degree of polyphagy. Historical records of ECB indicate that the species is polyphagous, consuming over 200 plant species

[4] E-mail: megorust@yahoo.com

(Hodgson 1928), and it is currently considered a pest on other crops including wheat, cotton, and numerous vegetables (Mason et al. 1996). A recent laboratory study of ECB also supports a wide diet breadth, with neonates preferentially feeding on various weed species over corn (Tate et al. 2006). However, studies commissioned by the EPA concluded that adult ECB could be produced from weeds and some grain crops, but in insufficient numbers to replace structured-refuge mandates (EPA 2001). Losey et al. (2001, 2002) also concluded that non-corn plants probably contribute little to adult populations of ECB.

Differences in reported feeding behaviors of ECB may be related to different races. There are two distinct pheromone races of ECB in the United States that utilize different pheromone blends of Z and E isomers of 11-tetradecyl-acetate (Carde et al. 1978). Since the two ECB pheromone races are partially isolated genetically (Dopman et al. 2005), Bt resistance may develop at different rates in the two races (Bontemps et al. 2004). It is possible that they differ in host-use patterns, and it is imperative to know how the two races utilize unstructured refuges for IRM. Research from France indicated that the E-race "hop–mugwort" ECB indeed had very different host utilization than Z-race ECB and generally segregated to non-corn hosts (Thomas et al. 2003, Bontemps et al. 2004). However, recent genetic (Malausa et al. 2007) and taxonomic (Frolov et al. 2007) studies indicate that the French hop–mugwort race is better characterized as another species, *Ostrinia scapulalis*, and that it is distinct from E-race *Ostrinia nubilalis*. Refuge utilization by E-race ECB in the United States remains largely unknown and previous research assessing ECB development in unstructured refuges did not distinguish pheromone races (EPA 2001, Losey et al. 2001). However, there is circumstantial evidence supporting differences in host utilization between races in the United States, with reports from North Carolina suggesting that the E race is more likely to infest cotton and potato than the Z race (Sorenson et al. 2005).

Here we use carbon stable-isotope analysis to assess the contribution of unstructured refuges to populations of E- and Z-race ECB. Plants that utilize the C_4 photosynthetic pathway sequester a significantly higher proportion of ^{13}C to ^{12}C, a ratio designated as $\delta^{13}C$, than plants utilizing the C_3 pathway. The $\delta^{13}C$ levels of an organism's tissues largely reflect the carbon signatures of its food, and thus can provide information on the feeding history of that individual (Deniro and Epstein 1978). Observations of ECB trapped in France and reared on diet derived from different plant material confirm that $\delta^{13}C$ analysis of adult ECB can distinguish unambiguously between a C_3 or C_4 larval host history (Ponsard et al. 2004, Malausa et al. 2007). In the northeastern United States, corn is by far the most common C_4 plant, though a small number of other C_4 agricultural crop and plant species also exist (Uva et al. 1997, Ponsard et al. 2004). Any ECB with a C_3-host

history must have developed on a non-corn host, and therefore in an unstructured refuge.

In addition to providing data on the proportions of E- and Z-race ECB development in unstructured refuges, we explore other ecological factors essential to the goals of IRM. We have identified physiological consequences for ECB developing in unstructured refuges that may reduce their fecundity relative to ECB developing in structured non-Bt corn refuges. Reduced fecundity would indicate that unstructured refuges may be less efficient than structured refuges in producing Bt-susceptible adults, which could increase the rate of resistance evolution (Ives and Andow 2002). We also have compiled extensive data on the geographic ranges of the two ECB pheromone races. If the two races exhibit different utilization of unstructured refuges and are not entirely sympatric, then adjusting IRM refuge requirements in different regions may be warranted.

MATERIALS AND METHODS

Isotope experiments

ECB (European corn borers) were collected weekly in upstate New York (USA) along the borders of sweet corn fields during 2006 in Scentry Heliothis traps (Scentry Biologicals, Billings, Montana, USA) baited with either E- or Z-race-specific Pherocon pheromone lures (Trécé, Adair, Oklahoma, USA). While there is a small error rate in E- and Z-race males responding to the pheromone blend typical of the opposite race (Linn et al. 1997), we assume that trap captures reflect the true race of the responding ECB. Random subsamples of moths from each sampling location were dried at 55°C for at least one week, and were weighed individually. The wings of 68 E-race and 71 Z-race ECB were each analyzed for $\delta^{13}C$ content using a Thermo Delta Plus isotope ratio mass spectrometer (IRMS) interfaced to a Carlo Erba NC2500 elemental analyzer (Thermo Fischer Scientific, Waltham, Massachusetts, USA) at the Cornell Isotope Laboratory (COIL; Cornell University, Ithaca, New York, USA) (Appendix A).

A second $\delta^{13}C$ stable-isotope experiment was conducted on all ECB ($n = 138$ E; 206 Z) collected on one farm in Penn Yan, New York, in 2006 to determine the relationships between adult mass, pheromone race, and host history. Moths were dried and weighed individually. Moths from the "large" and "small" tails of the mass distributions of each pheromone race were selected for isotope analysis. The masses of individuals of each race were matched within size classes so that the mean masses of each category would be as similar as possible. We analyzed 11 "large" E (9.83 ± 3.66 mg [mean ± SD]), 11 "large" Z (10.92 ± 1.97 mg), 7 "small" E (3.92 ± 0.24 mg), and 9 "small" Z moths (3.78 ± 0.37 mg). Additionally, the four smallest moths collected (2.05 ± 0.13 mg), which were all E race, were analyzed for $\delta^{13}C$ content. Isotope analysis was conducted as described previously.

The $\delta^{13}C$ signatures were converted to categories of host history by scoring individuals with $\delta^{13}C$ values less than $-20‰$ as having a C_3 host history, while $\delta^{13}C$ values greater than $-15‰$ were scored as having a C_4 host history (Deniro and Epstein 1978, Ponsard et al. 2004). For the first isotope experiment, a Fischer exact test was used to assess the relationship between pheromone race and ECB host history. The relationship between sampling location and ECB host history was assessed for the E race, using likelihood-ratio tests of chi-square estimates. The effects of host history and pheromone race on moth dry mass were tested using a standard least-squares analysis of variance (ANOVA) of a full factorial model. Contrasts were made to test the specific hypotheses that E- and Z-race ECB weigh the same when they develop on C_4 plants, and that each race weighs less when they develop on C_3 plants (SAS Institute 2007). For the second isotope experiment, the effects of moth size category (large and small) and pheromone race on host history were analyzed with an exact logistic regression using Stata 10.0 software (StataCorp 2008). The relationship between pheromone race and host history within the different size categories was further investigated using Fischer exact tests of two-by-two contingency tables (SAS Institute 2007).

Female size and fecundity

We examined the relationship between size and lifetime egg production of Z-race ECB reared at the USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, Iowa, USA. The ECB colony was established in June 2007 from wild adults captured in light traps in central Iowa, and three cohorts representing three generations ($N = 66$, 37, and 60 females) were tested during January–April 2008. Insects were reared at $27°C$, 16 h:8 h (light:dark), and 80% relative humidity, following standard procedures (Guthrie et al. 1985). Eggs were heat-treated to ensure a disease-free colony (Raun 1961).

Female pupae were weighed to the nearest milligram on the second day after pupation. Pupae were held separately in small plastic cups and checked daily for adult emergence. On the day of eclosion, each female was transferred to a small wire-mesh cage (8.5 cm diameter, 6 cm tall) containing two, 2–5 day-old males for mating (Kira et al. 1969). The males remained in the cage throughout the life of the female, and were replaced with fresh males if the earlier males died. The mesh on the lid was wide enough to allow females to oviposit through it onto a wax paper disc held in place by the upper half of a glass petri dish. The wax sheet was changed daily and the eggs were allowed to develop for two days. Fertile and infertile eggs were distinguished by color change associated with embryo development and were counted under a dissecting microscope. Total eggs laid, including both fertile and non-fertile from females laying >50% fertile eggs, was used in analysis. The effect of female pupal size on fecundity was assessed by linear regression of total lifetime eggs laid on female pupal mass (Analytical Software 2000).

Geographic distributions

ECB data from New England and Mid-Atlantic states were obtained from the Pest Watch database (Fleischer 2008). Data from North Carolina were obtained from Sorenson et al. (2005), data from South Carolina were obtained from Durant et al. (1986), data from Ohio were contributed by C. Welty (*unpublished data*), and data from Iowa were contributed by D. Sumerford and T. Sappington (*unpublished data*). ECB data were consolidated by summing all E and Z moths collected over all years of record per county before further analyses (Appendix B).

Spatial information about ECB distributions and agronomic data were compiled and maps were created in Manifold System 8.0 geographic information systems (GIS) software (Manifold System 2008). Data on corn and vegetable acreages (including sweet corn) per county in the Pest Watch database and North Carolina, Ohio, and South Carolina were obtained from the 2002, 1992, and 1987 Census of Agriculture, respectively (USDA 2008b). The relationships between the proportion of E moths per county and longitude, field corn acreage, and vegetable acreage were analyzed using multiple regression (SAS Institute 2007). Prior to analysis, corn and vegetable acreages per county were normalized by the total area of each county. Data from Iowa were excluded from this analysis to avoid skewing the results with an outlier data point. ANOVA was used to test whether the percentages of ECB that were E race were higher in counties in the eastern (east of the Hudson River: Maine, New Hampshire, Vermont, Massachusetts, Connecticut, and Long Island, NY), than western (west of the Hudson River: Pennsylvania, Maryland, Delaware, upstate New York) regions of the Pest Watch data set (SAS Institute 2007).

RESULTS

Isotope experiments

All moths sampled had either a $\delta^{13}C$ isotope signature reflecting larval feeding on C_4 ($N = 160$ moths; mean $= -12.198‰$, maximum $= -10.254‰$, minimum $= -14.638‰$) or C_3 ($N = 26$ moths; mean $= -27.901‰$, max $= -23.752‰$, min $= -31.041‰$) plants. Among the moths sampled, there was no evidence that any had a mixed diet of C_3 and C_4 plants as larvae (Appendix C).

E- and Z-race European corn borers (ECB) utilized non-corn hosts at different rates (Fischer's exact two-tail test; $N = 139$ moths, $P = 0.013$). Approximately 18% of the E-race adults captured in pheromone traps throughout upstate New York developed as larvae on C_3 plants (12 of 68 moths) compared to only 4% of captured Z-race moths (3 of 71 moths). However, rates of utilizing unstructured refuges were spatially very variable for the E race, ranging from 0% to 40%. Indeed, location significantly affected the rate that E-race moths devel-
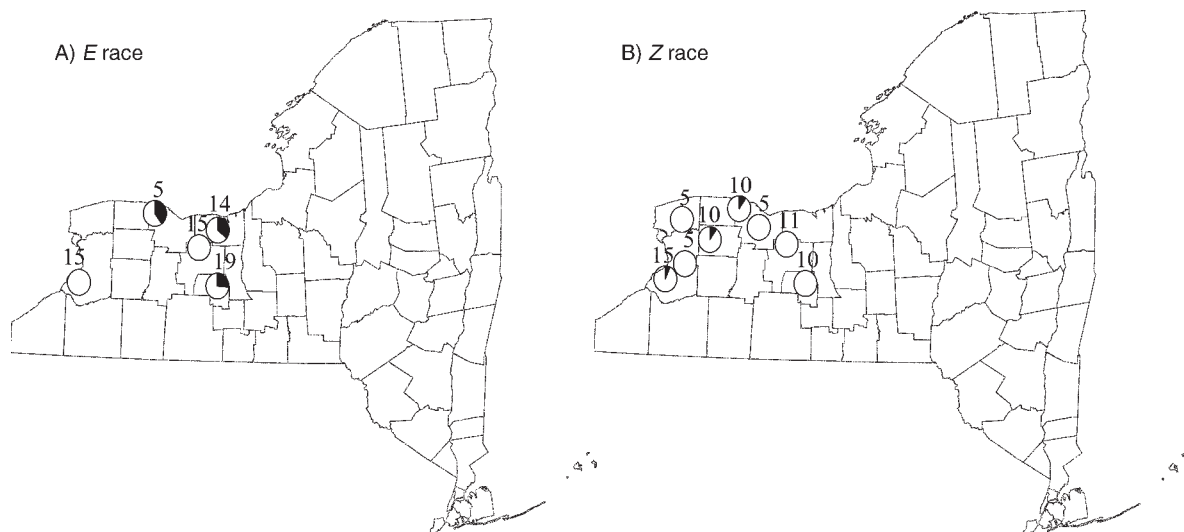
FIG. 1. Pie charts show proportions of adult European corn borer (ECB) moths collected in pheromone traps in upstate New York, USA, specific to (A) the E race or (B) the Z race whose $\delta^{13}C$ signatures reflect larval feeding on C_3 (unstructured refuges; black) and C_4 (white) plants in upstate New York. The numbers above the pie charts are sample sizes.

oped in unstructured refuges ($\chi^2 = 16.496$, df $= 4$, $P = 0.0024$) (Fig. 1).

Larval host plant had a significant effect on adult mass of E-race ECB. There was no significant difference in adult mass between the E and Z race when they developed on C_4 plants ($F = 3.69$, df $= 1$, 91, $P = 0.06$). However, the masses of E-race ECB were significantly less when they developed on C_3 hosts (4.72 ± 2.33 mg [mean $\pm$ SD]) than on C_4 hosts (6.16 ± 1.53 mg) ($F = 6.33$, df $= 1,91$, $P = 0.01$). In contrast, there did not appear to be a decrease in mass for Z-race ECB that developed in unstructured refuges ($F = 0.59$, df $= 1$, 91, $P > 0.5$), but there were only two Z-race ECB to test in this category (Fig. 2).

The second isotope experiment further supports a relationship between ECB pheromone race, adult mass, and host history (Table 1). Small moths were significantly more likely to have developed on a C_3 plant than large moths (odds ratio $= 92.577$, 95% CI $= 3.5$ to infinity, $P < 0.0001$). Also, E-race moths were significantly more likely to have developed on a C_3 plant than were Z-race moths (odds ratio $= 34.168$, 95% CI $= 8.8$ to infinity; $P = 0.0014$). Differences in host history between the two ECB races are especially apparent in small moths where the E race is much more likely than the Z race to have developed on C_3 plants (Fischer's exact one-tail test, $N = 16$ moths, $P = 0.0007$). The four smallest E-race ECB, which were the smallest of all the moths sampled at Penn Yan, New York, all had $\delta^{13}C$ signatures indicative of a C_3 host history.

Female size and fecundity

Lifetime egg production was positively related to female size, as indexed by pupal mass ($F = 2.56$, df $= 1$, 161, $P < 0.0001$). Female pupal mass explained 25% of

the variation in lifetime egg production. Within the range of pupal masses examined, our regression model predicts that a threefold increase in pupal mass corresponds to a 3.5-fold increase in the number of eggs an ECB female will lay (Fig. 3). Lifetime percent fertile eggs laid per female averaged 86.6 ± 0.08 eggs (mean $\pm$ SE).

Geographic distributions

On a countywide scale, Z- and E-race ECB always co-occurred at the locations sampled in this study, except in Iowa where moths responded only to the Z pheromone (Fig. 4). However, the proportion of E moths expected in a county is significantly related to longitude,
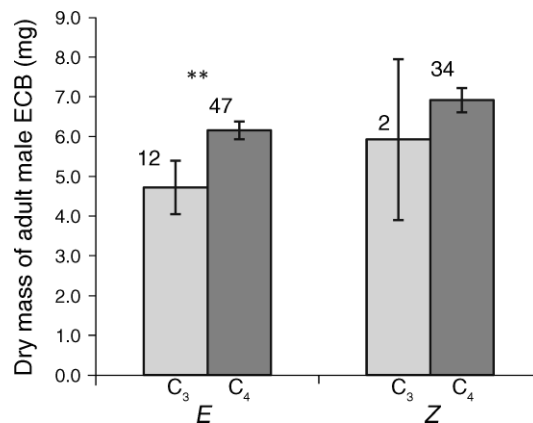

FIG. 2. Dry mass of adult male ECB collected in E and Z pheromone-baited traps in upstate New York. Bars show mass (mean $\pm$ SE) of ECB that developed on C_3 and C_4 hosts. Numbers above the bars indicate sample sizes. Asterisks denote a significant difference ($P = 0.01$) in the mass of E moths that fed on C_3 vs. C_4 plants.

TABLE 1. Numbers of E- and Z-race adult male European corn borer (ECB) moths of two size categories collected in pheromone traps in Penn Yan, New York, USA, that had $\delta^{13}C$ signatures characteristic of either C_3 or C_4 larval host plants.

		$\delta^{13}C$ signature	
ECB race	Insect size	C_3	C_4
E	large	0	11
	small	7	0
Z	large	0	11
	small	1	8

increasing from west to east ($F = 21.04$, df = 1, 83, $P < 0.0001$). Counties east of Pennsylvania and upstate New York reported an average of 59% E moths, which is significantly more than the average 23% E race reported in the western Pest Watch region ($F = 54.61$, df = 1, 73, $P < 0.0001$). In addition to longitude, land-use also appears to play a role in the distribution of ECB races. The proportion of E-race ECB per county is positively related to non-corn acreage ($F = 4.98$, df = 1, 83, $P = 0.028$). Vegetable acreage was not significantly related to the distribution of E moths ($F = 0.15$, df = 1, 83, $P > 0.5$).

DISCUSSION

The differences in host use between E- and Z-race ECB found in this study have important implications for insect-resistance management (IRM) in Bt-corn (Gould 1998). Relatively high proportions of E-race ECB developing on species other than corn indicate that unstructured refuges may contribute to slowing the development of resistance to Bt in the E race. In contrast, our results indicate that only a very low proportion of Z-race ECB develop in unstructured refuges in upstate New York (USA), and that unstructured refuges probably contribute relatively little to preventing Bt-resistance evolution in this race. The average 4% of Z-race ECB that developed in unstructured refuges in this study concurs with data from France where only 3% of Z-race Ostrinia nubilalis collected in pheromone traps had developed on C_3 hosts (Bontemps et al. 2004). Furthermore, since "mistaken" attraction to the lure of the opposite race can happen, especially in the case of E males responding to the Z pheromone blend (Linn et al. 1997), the proportion of Z-race ECB developing in unstructured refuges determined in our study could be an overestimate.

Previous research concluding that unstructured refuges are unlikely to contribute to adult populations of ECB may have been working primarily with Z-race ECB, even though the race was not identified. This is certainly true for studies in the Midwestern United States where the E race presumably is not present (EPA 2001; Fig. 3). Additionally, research by Losey et al. (2001, 2002) on unstructured refuges and ECB was conducted in only one region in upstate New York and

one in Pennsylvania. While both pheromone races are present in these regions (Fig. 3), farm-to-farm variability in the proportional abundance of E moths can be quite high (Sorenson et al. 2005), and the contribution by the E race was undetermined.

Taken as a whole, our results do not support a relaxing of structured-refuge requirements for ECB in Bt corn. While our isotope survey shows that E-race ECB develop in unstructured refuges at relatively high rates in New York, and correlations between non-corn habitat and E-race ECB confirm that wide host utilization may occur throughout its range, other results indicate that adjusting structured-refuge requirements for Bt corn would be unwarranted. First, there seems to be a large amount of spatial variability in the proportions of E-race ECB feeding on non-corn hosts. Second, adult masses of ECB appear to be reduced by feeding on C_3 hosts. Reduced mass of ECB females, in turn, was related to lower fecundity. Because low-mass moths produce fewer lifetime eggs, the effective refuge size of non-corn hosts likewise is reduced and could increase rates of resistance evolution (Gould 1998, Ives and Andow 2002, Gustafson et al. 2006). Finally, our compilation of E- and Z-race ECB distributions indicates that the Z-race's range fully overlaps with that of the E race in the United States at a countywide scale. Where there is sympatry, IRM strategies should be conservatively based on the Z race, for which non-corn hosts appear to produce insufficient numbers of susceptible adults to serve as reliable unstructured refuges.

Our research showing different rates of unstructured-refuge utilization between ECB pheromone races also has important implications for IRM in Bt cotton. Surveys of the major U.S. cotton pests, Heliothis virescens and Helicoverpa zea, indicate that both species utilize unstructured refuges at higher rates than we observed for ECB (Gould et al. 2002, Gustafson et al. 2006, Orth et al. 2007, Jackson et al. 2008). These data have played important roles in the 2007 approval by EPA to eliminate structured-refuge requirements in
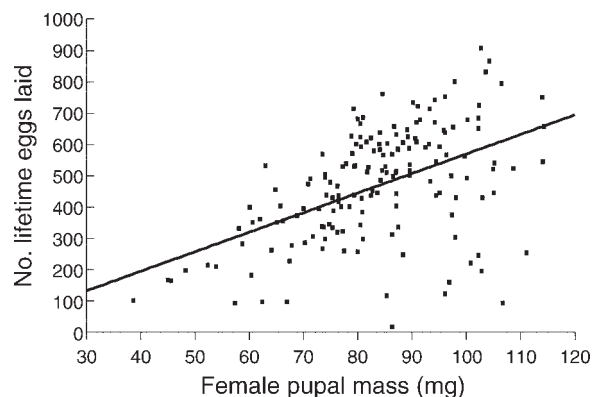

FIG. 3. Linear regression of lifetime eggs laid vs. female pupal mass for laboratory-reared Z-race European corn borers.

Fig. 4. Pie charts show proportions of *E* (black) and *Z* (white) pheromone races of ECB in the eastern and central United States based on captures in pheromone traps. See Appendix A for sources and details of samples.

IRM plans for Bt cotton varieties pyramided with Cry1Ac and Cry2Ab2 genes in parts of the United States (EPA 2006*a*, 2008). However, as with IRM plans for *O. nubilalis*, differences in host utilization between possible races of cotton pests have not been carefully considered. Research indeed points to the possibility of pheromone races within *Helicoverpa armigera*, the Old World sister species of *H. zea* (Tamhankar et al. 2003, Cho et al. 2008), and to variation in pheromone composition and male response in *Heliothis virescens* (Groot et al. 2009). Given the large differences we observed for unstructured refuge utilization between *O. nubilalis* pheromone races and the potential impacts on Bt-resistance evolution and IRM, more research into possible races of cotton pests appears warranted.

In addition to its implications for IRM, differences in feeding behaviors between *E*- and *Z*-race ECB are important for integrated pest management (IPM) in vegetable crops. Since vegetable processors have very low tolerances for ECB contamination, insecticides targeting ECB are often applied preventively based on ECB flight activity and plant growth stage (Mason et al. 1996). However, our data indicate that adult ECB that have fed on C$_3$ plants, including vegetables, are predominantly *E* race. This result raises questions about

the role of *Z*-race ECB in vegetable systems other than sweet corn. It is unclear whether *Z*-race ECB lay eggs on vegetables but their larvae have low survival to adulthood, or whether the small percentage of *Z*-race ECB that feed on C$_3$ plants is sufficient to cause economic losses in vegetables. Alternatively, *E*-race ECB may constitute the major contributor to vegetable damage. If so, IPM programs for managing ECB in vegetables other than sweet corn should be focused on the flight activity of *E* moths, which is not necessarily in phase with that of *Z* moths (Sorenson et al. 2005). An increased focus on the biology and ecology of the *E* race could improve pest management and reduce insecticide use in vegetable crops.

This research also provides general insight into the ecological differences between the ECB races. We have shown through isotope analysis that *E* and *Z* moths have different host-use patterns in New York. The large-scale geographic relationship that we found between non-corn habitat and proportions of *E*-race ECB further indicates that development in unstructured refuges by *E*-race ECB may be a general phenomenon throughout its range. Our distribution map of ECB pheromone races also shows clear differences in their ranges. The underlying reasons for the strong longitudinal trend
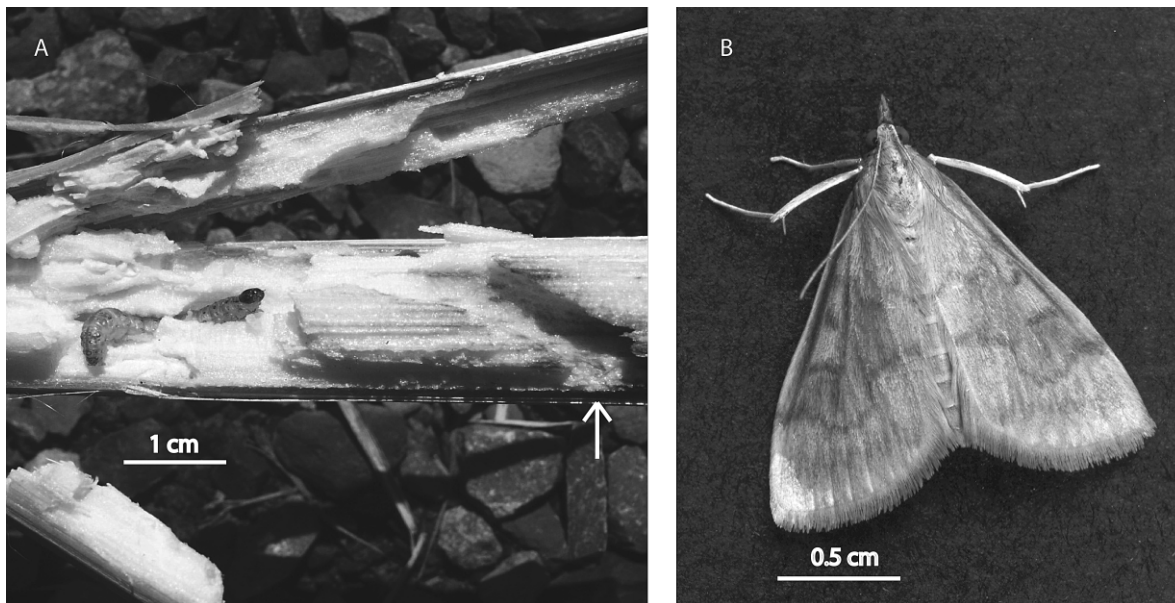
PLATE 1. European corn borer (*Ostrinia nubilalis*). (A) Larva and tunneling damage to corn (*Zea mays*), indicated by the white arrow; (B) adult female. Photo credits: (A) M. E. O'Rourke, (B) T. W. Sappington.

are unknown, but a variety of hypotheses can be posited. The *E* race was not recognized until 1972 (Roelofs et al. 1972) and its reduced range may simply be the result of its introduction after the *Z* race, since multiple introductions of ECB into the United States have been acknowledged (Brindley and Dicke 1963). Alternatively, the *E* race may be more ecologically constrained in North America than the *Z* race. They may be poorer dispersers, resulting in slower range expansion, or less adapted to the climate in the midwestern United States.

Ecological differences between the pheromone races, along with evidence for restricted gene flow and independent evolution (Dopman et al. 2005) emphasize that ECB races should be clearly identified in future research. This is especially true in the New England states where we found that the *E* race often predominates. In addition to pheromone races, there are genetically distinct voltinism races that utilize the *Z* pheromone blend and differ in post-diapause development times (Coates et al. 2004, Dopman et al. 2005). Although diapause response has been extensively studied among voltinism races (Calvin and Song 1994), little is known about the distributions or behaviors of the univoltine race. As with the pheromone races, they likely have unique evolutionary trajectories and should not be ignored. In our research, since uni- and bivoltine *Z*-race ECB are known to exist sympatrically in New York (Glover et al. 1991), $\delta^{13}C$ samples likely included both voltinism races. However, their proportions in samples are unknown and we cannot definitively conclude that both *Z* voltinism races use unstructured refuges at very low rates.

All the different races of ECB make the taxonomy of the species difficult. Until recently, studies from France indicated that *E*-race ECB infested mainly hop and mugwort (Thomas et al. 2003, Bontemps et al. 2004). However, Malausa et al. (2007) suggested the degree of reproductive isolation between the *Z*- and *E*-race ECB in France was high enough to justify species status. Frolov et al. (2007) concluded that the *E*-race populations in France actually belong to the sibling species *Ostrinia scapulalis*, though the existence of *E*-race *O. nubilalis* in other parts of Europe is still recognized. While we found that *E*-race ECB from New York consumed non-corn plants at an average rate of 18%, this is very different from the *E*-race "hop–mugwort" species (*O. scapulalis*) in France that infests C_3 plants at a rate of 86% (Bontemps et al. 2004). Thus, our $\delta^{13}C$ data on the host history of *E*-race ECB in New York support the idea that they are *E*-race *O. nubilalis* rather than *E*-race hop–mugwort *O. scapulalis* studied in France. However, the possibility remains that there is assortative mating within the *E* race between those that feed on corn and non-corn hosts, or that there is a combination of *E* races of *O. nubilalis* and *O. scapulalis* within the United States.

This research has shown that utilization of unstructured refuges differs between the *E* and *Z* pheromone races of ECB in the United States, with important implications for IRM and IPM plans. Where multiple races of a species have overlapping distributions, IRM strategies should be conservatively based on the race most likely to develop resistance, in this case the *Z* race of ECB. This, of course, requires not only that we can identify the cryptic races, but also that we understand the basic ecological, behavioral, and evolutionary differences between them. While unraveling the differences among members of race or species complexes can

be difficult, we have shown how understanding them can have important applied economic and policy implications.

LITERATURE CITED

Analytical Software. 2000. Statistix for Windows: user's manual. Analytical Software, Tallahassee, Florida, USA.

Andow, D. A. 2002. Resisting resistance to Bt-corn. Pages 99–125 *in* D. K. Letourneau and B. E. Burrows, editors. Genetically engineered organisms: assessing environmental and human health effects. CRC Press, New York, New York, USA.

Andow, D. A., and C. Zwahlen. 2006. Assessing environmental risks of transgenic plants. Ecology Letters 9:196–214.

Bickford, D., D. J. Lohman, N. S. Sodhi, P. K. L. Ng, R. Meier, K. Winker, K. K. Ingram, and I. Das. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution 22:148–155.

Bontemps, A., D. Bourguet, L. Pelozuelo, M. T. Bethenod, and S. Ponsard. 2004. 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. Proceedings of the Royal Society of London B 271:2179–2185.

Brindley, T. A., and F. F. Dicke. 1963. Significant developments in European corn borer research. Annual Review of Entomology 8:155–176.

Calvin, D. D., and P. Z. Song. 1994. Variability in postdiapause development periods of geographically separate *Ostrinia nubilalis* (Lepidoptera, Pyralidae) populations in Pennsylvania. Environmental Entomology 23:431–436.

Carde, R. T., W. L. Roelofs, R. G. Harrison, A. T. Vawter, P. F. Brussard, A. Mutuura, and E. Munroe. 1978. European corn borer: pheromone polymorphism or sibling species? Science 199:555–556.

Cho, S., A. Mitchell, C. Mitter, J. Regier, M. Matthews, and R. Robertson. 2008. Molecular phylogenetics of heliothine moths (Lepidoptera: Noctuidae: Heliothinae), with comments on the evolution of host range and pest status. Systematic Entomology 33:581–594.

Coates, B. S., D. V. Sumerford, and R. L. Hellmich. 2004. Geographic and voltinism differentiation among North American *Ostrinia nubilalis* (European corn borer) mitochondrial cytochrome *c* oxidase haplotypes. Journal of Insect Science 4(35):1–9.

Deniro, M. J., and S. Epstein. 1978. Influence of diet on distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

Dopman, E. B., L. Perez, S. M. Bogdanowicz, and R. G. Harrison. 2005. Consequences of reproductive barriers for genealogical discordance in the European corn borer. Proceedings of the National Academy of Sciences (USA) 102:14706–14711.

Durant, J. A., D. G. Manley, and R. T. Carde. 1986. Monitoring of the European corn borer (Lepidoptera, Pyralidae) in South Carolina using pheromone traps. Journal of Economic Entomology 79:1539–1543.

EPA [U.S. Environmental Protection Agency]. 2001. Biopesticides Registration Action Document. *Bacillus thuringiensis* (*Bt*) plant-incorporated protectants. U.S. Environmental Protection Agency, Washington, D.C., USA. ⟨http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm⟩

EPA [U.S. Environmental Protection Agency]. 2006a. BPPD review of data and support materials submitted by Monsanto to amend the insect resistance management requirements for Bollgard II Bt cotton. Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C., USA. ⟨http://www.epa.gov/scipoly/sap/meetings/2006/june/bppd-rev1a.pdf⟩

EPA [U.S. Environmental Protection Agency]. 2006b. Insect resistance management fact sheet for *Bacillus thuringiensis* (Bt) corn products. U.S. Environmental Protection Agency, Washington, D.C., USA. ⟨http://www.epa.gov/oppbppd1/biopesticides/pips/bt_corn_refuge_2006.htm⟩

EPA [U.S. Environmental Protection Agency]. 2008. Insect resistance management fact sheet for *Bacillus thuringiensis* (*Bt*) cotton products. U.S. Environmental Protection Agency, Washington, D.C., USA. ⟨http://www.epa.gov/oppbppd1/biopesticides/pips/bt_cotton_refuge_2006.htm⟩

Ferrari, J., H. C. J. Godfray, A. S. Faulconbridge, K. Prior, and S. Via. 2006. Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. Evolution 60:1574–1584.

Fleischer, S. 2008. Pest watch. Pennsylvania State University, University Park, Pennsylvania, USA. ⟨http://www.pestwatch.psu.edu⟩

Frolov, A. N., D. Bourguet, and S. Ponsard. 2007. Reconsidering the taxomony of several *Ostrinia* species in the light of reproductive isolation: a tale for Ernst Mayr. Biological Journal of the Linnean Society 91:49–72.

Glover, T. J., J. J. Knodel, P. S. Robbins, C. J. Eckenrode, and W. L. Roelofs. 1991. Gene flow among three races of European corn borers (Lepidoptera, Pyralidae) in New York State. Environmental Entomology 20:1356–1362.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annual Review of Entomology 43:701–726.

Gould, F. 2000. Testing Bt refuge strategies in the field. Nature Biotechnology 18:266–267.

Gould, F., N. Blair, M. Reid, T. L. Rennie, J. Lopez, and S. Micinski. 2002. *Bacillus thuringiensis*-toxin resistance management: stable isotope assessment of alternate host use by *Helicoverpa zea*. Proceedings of the National Academy of Sciences (USA) 99:16581–16586.

Groot, A. T., O. Inglis, S. Bowdridge, R. G. Santangelo, C. Blanco, J. D. Lopez, A. T. Vargas, F. Gould, and C. Schal. 2009. Geographic and temporal variation in moth chemical communication. Evolution 63:1987–2003.

Gustafson, D. I., G. P. Head, and M. A. Caprio. 2006. Modeling the impact of alternative hosts on *Helicoverpa zea* adaptation to Bollgard cotton. Journal of Economic Entomology 99:2116–2124.

Guthrie, W. D., J. C. Robbins, and J. L. Jarvis. 1985. *Ostrinia nubilalis*. Pages 407–413 *in* P. Singh and R. F. Moore, editors. Handbook of insect rearing. Elsevier, New York, New York, USA.

Hodgson, B. E. 1928. The host plants of the European corn borer in New England. Technical Bulletin 77. United States Department of Agriculture, Washington, D.C., USA. ⟨http://www.nal.usda.gov/ref/USDApubs/tb.htm#sortnbr⟩

Ives, A. R., and D. A. Andow. 2002. Evolution of resistance to Bt crops: directional selection in structured environments. Ecology Letters 5:792–801.

Jackson, R. E., et al. 2008. Regional assessment of *Helicoverpa zea* populations on cotton and non-cotton crop hosts. Entomologia Experimentalis et Applicata 126:89–106.

James, C. 2007. Global status of commercialized biotech/GM crops. ISAAA, Cornell University, Ithaca, New York, USA.

Kira, M. T., W. D. Guthrie, and J. L. Huggans. 1969. Effect of drinking water on production of eggs by European corn borer. Journal of Economic Entomology 62:1366–1370.

Linn, C. E., M. S. Young, M. Gendle, T. J. Glover, and W. L. Roelofs. 1997. Sex pheromone blend discrimination in two races and hybrids of the European corn borer moth, *Ostrinia nubilalis*. Physiological Entomology 22:212–223.

Losey, J. E., D. D. Calvin, M. E. Carter, and C. E. Mason. 2001. Evaluation of noncorn host plants as a refuge in a resistance management program for European corn borer (Lepidoptera: Crambidae) on Bt-corn. Environmental Entomology 30:728–735.

Losey, J. E., M. E. Carter, and S. A. Silverman. 2002. The effect of stem diameter on European corn borer behavior and survival: potential consequences for IRM in Bt-corn. Entomologia Experimentalis et Applicata 105:89–96.

Malausa, T., A. Dalecky, S. Ponsard, P. Audiot, R. Streiff, Y. Chaval, and D. Bourguet. 2007. Genetic structure and gene flow in French populations of two *Ostrinia* taxa: host races or sibling species? Molecular Ecology 16:4210–4222.

Manifold System. 2008. Geographic information systems user manual. Manifold system 8.0. Manifold Net, Carson City, Nevada, USA.

Mason, C., M. Rice, D. Calvin, J. Van Duyn, W. Showers, W. Hutchison, J. Witkowski, R. Higgins, D. Onstad, and G. Dively. 1996. European corn borer ecology and management. North Central Regional Extension publication number 28. Iowa State University, Ames, Iowa, USA.

Orth, R. G., G. Head, and M. Mierkowski. 2007. Determining larval host plant use by a polyphagous lepidopteran through analysis of adult moths for plant secondary metabolites. Journal of Chemical Ecology 33:1131–1148.

Ponsard, S., M. T. Bethenod, A. Bontemps, L. Pelozuelo, M. C. Souqual, and D. Bourguet. 2004. Carbon stable isotopes: a tool for studying the mating, oviposition, and spatial distribution of races of European corn borer, *Ostrinia nubilalis*, among host plants in the field. Canadian Journal of Zoology 82:1177–1185.

Qiao, F., J. Wilen, and S. Rozelle. 2008. Dynamically optimal strategies for managing resistance to genetically modified crops. Journal of Economic Entomology 101:915–926.

Raun, E. S. 1961. Elimination of microsporidiosis in laboratory-reared European corn borers by use of heat. Journal of Insect Pathology 3:446–448.

Roelofs, W. L., R. T. Carde, R. J. Bartell, and P. G. Tierney. 1972. Sex attractant trapping of the European corn borer in New York. Environmental Entomology 1:606–608.

SAS Institute. 2007. JMP. Statistics and graphics guide. SAS Institute, Cary, North Carolina, USA.

Sorenson, C. E., G. G. Kennedy, C. Schal, and J. F. Walgenbach. 2005. Geographical variation in pheromone response of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), in North Carolina: a 20-year perspective. Environmental Entomology 34:1057–1062.

StataCorp. 2008. Stata statistical software release 10 user manual. StataCorp, College Station, Texas, USA.

Tamhankar, A. J., T. P. Rajendran, N. H. Rao, R. C. Lavekar, P. Jeyakumar, D. Monga, and O. M. Bambawale. 2003. Variability in response of *Helicoverpa armigera* males from different locations in India to varying blends of female sex pheromone suggests male sex pheromone response polymorphism. Current Science 84:448–450.

Tate, C. D., R. L. Hellmich, and L. C. Lewis. 2006. Evaluation of *Ostrinia nubilalis* (Lepidoptera: Crambidae) neonate preferences for corn and weeds in corn. Journal of Economic Entomology 99:1987–1993.

Thomas, Y., M. T. Bethenod, L. Pelozuelo, B. Frerot, and D. Bourguet. 2003. Genetic isolation between two sympatric host-plant races of the European corn borer, *Ostrinia nubilalis* Hubner. I. sex pheromone, moth emergence timing, and parasitism. Evolution 57:261–273.

USDA [U.S. Department of Agriculture]. 2008a. Acreage. U.S. Department of Agriculture, Washington, D.C., USA. ⟨http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2008.pdf⟩

USDA [U.S. Department of Agriculture]. 2008b. National Agricultural Statistics Service. U.S Census of Agriculture. U.S. Department of Agriculture, Washington, D.C., USA. ⟨http://www.agcensus.usda.gov/⟩

Uva, R. H., J. C. Neal, and J. M. DiTomaso. 1997. Weeds of the Northeast. Cornell University Press, Ithaca, New York, USA.

APPENDIX A

Proportions of *E*- and *Z*-race ECB sampled from upstate New York that developed on C_3 hosts according to $\delta^{13}C$ analyses (*Ecological Archives* A020-042-A1).

APPENDIX B

Total number of *E*- and *Z*-race moths trapped, the number of trap locations monitored, and the duration of trapping for each county mapped in Fig. 4 (*Ecological Archives* A020-042-A2).

APPENDIX C

Histogram of $\delta^{13}C$ values of all ECB analyzed (*Ecological Archives* A020-042-A3).