Geographical Variation in Pheromone Response of the European Corn Borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), in North Carolina: A 20-Y Perspective

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ABSTRACT Pheromone traps were used to assess the distribution of two pheromone races of European corn borer, *Ostrinia nubilalis* (Hübner), in North Carolina, ≈10 and 20 yr after previous, similar assessments. In the previous studies, moths responding to a 97*Z*: 3*E* isomeric blend (*Z* blend) of 11-tetradecenyl acetate predominated in the far western parts of the state, whereas moths responding to a 3*Z*: 97*E* blend (*E* blend) prevailed in the east, with a substantial zone of overlap occurring in the eastern Piedmont. There was evidence that the *E* responsive population had expanded westward between 1978 and 1988. In this study, the distribution of the two races seemed to remain essentially unchanged from that observed in the late 1980s, and no evidence of a continued westward expansion of *E* responsive moths was detected.

KEY WORDS pheromone race distribution, landscape effects, pheromone trapping, cone trap

THE EUROPEAN CORN BORER, *Ostrinia nubilalis* (Hübner), is a well-known, serious pest of maize (*Zea mays* L.) and several other crops. Populations in North America apparently originated from several different introductions and are polymorphic with respect to the pheromone produced by calling females. Females produce either a 97%*Z*:3%*E* (*Z* strain) isomeric ratio of 11-tetradecenyl acetate (11-TDA) or a 3%*Z*:97%*E* (*E* strain) ratio (Cardé et al. 1975, Kochansky et al. 1975); F_1 hybrids between these two strains produce a 35*Z*:65*E* percent blend (Klun and Maini 1979). Both pheromone strains are found in North Carolina.

Kennedy and Anderson (1980), working in Johnston and Wake Counties in the eastern edge of the Piedmont district of North Carolina and using delta-style sticky pheromone traps, captured significantly more moths in Z-baited traps than in *E*-baited traps or traps baited with two intermediate blends. Subsequently, Sorenson et al. (1992), using a modified Harstack wire-cone trap design, determined that *E* strain moths predominated in the eastern, coastal plain part of North Carolina, whereas virtually all captures in the far western parts of the state were by Z-baited traps. They also determined that there was a substantial zone, typified by Wake and Franklin Counties, where both strains occurred; within this zone, the proportions of the population responding to Z and *E* pheromone blends were extremely dynamic both spatially and temporally. In that study, a significantly greater proportion of moths captured in Wake County responded to a hybrid (35*Z*:65*E*) blend of pheromone than in any of the other counties to the east or west. Unlike the earlier study in the same area (Kennedy and Anderson 1980), Sorenson et al. (1992) measured slightly greater response to *E* strain lures in Wake County than to Z strain lures, suggesting a westward expansion of the *E* strain.

We conducted additional trapping studies, ≈10 yr after the study of Sorenson et al. (1992), to determine if the distribution of the *Z* and *E* strains, as measured by pheromone trap captures, had changed.

Materials and Methods

Lures used in these studies were prepared in the laboratory. Both isomers of 11-tetradecenyl acetate (Z and *E* 11–14:OAc; 11-TDA) were obtained from Sigma (St. Louis, MO). Stock solutions were made in *n*-hexane (Fisher, Pittsburgh, PA), and the ratio of *E*/*Z* isomers and their concentrations were confirmed (each was 97% pure) by gas chromatography (GC). Working solutions of 97:3 *Z*:*E* and 3:97 *Z*:*E* were made at a concentration of 2 μg/μl hexane and again confirmed by GC. Samples were injected into a splitless injector in a HP5890 gas chromatograph and separated in a 50 m by 0.31 mm by 0.25 μm Carbowax 20M column (Supelco, Bellefonte, PA) that was temperature programmed from 80 (with a 2-min hold) to 220°C at 10°C/min. The injector and FID were held at 230 and 240°C, respectively. Helium was used as carrier gas at a flow rate of 30 cm/s.

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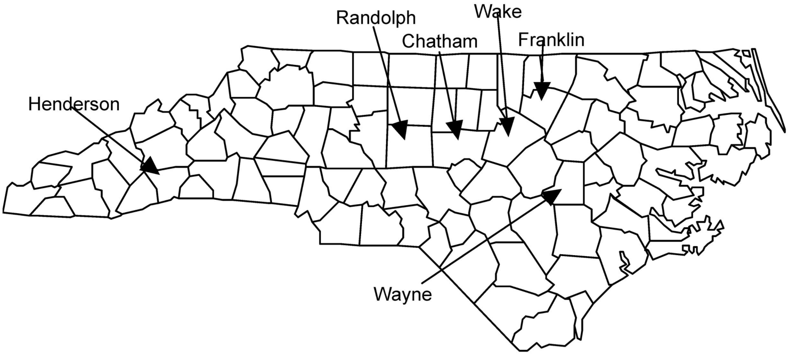

Fig. 1. Locations of North Carolina counties included in European corn borer pheromone trapping studies, 1998–2000.

Red rubber septa (Thomas Scientific, Swedesboro, NJ) were extracted with dichloromethane three times for 1 h each, air-dried for 48 h in a fume hood, and loaded with 50 μl (100 μg) of the appropriate solution. The septa were air-dried in a fume hood and stored at -20°C until deployed in the field.

Wire cone traps identical to those used by Sorenson et al. (1992) were deployed in Chatham, Franklin, Henderson (and adjoining portions of Buncombe), Randolph, Wayne, and Wake Counties in North Carolina (Fig. 1). All of these areas were included in the previous study during the years 1985–1988. Wayne County lies in the inner Coastal Plain, and in the previous study, *E* strain captures predominated. Chatham and Randolph Counties lie in the Piedmont region, whereas Henderson County is in the Appalachian Mountains; *Z* strain captures predominated in all these counties in the previous study. Wake and Franklin Counties lie in the fall zone, an area of transition between the Piedmont and the coastal plain; in the previous study, both *E* and *Z* strain captures were common, with a slight bias toward *E* race captures. Traps were placed in each county for one to three growing seasons.

Within each county, three commercial maize fields were identified; each field served as a replicate. Within each field, three traps were deployed. Traps were sited along one edge of the field in the grassy border vegetation and were spaced at least 30 m apart. Within each replicate during any given sampling interval, one trap was baited with a 97%*Z*:3%*E* 11-TDA lure, one trap was baited with a 3%*Z*:97%*E* 11-TDA lure, and the third was left unbaited as a control. Lures were placed on the traps by means of a small binder clip (Charles Leonard No. 2, Charles Leonard Inc., Glendale, NY) hung on a nail in the upright portion of the trap so that the lure faced away from the support and was located ≈8 cm above the lower rim of the screen wire trap cone. Each lure was identified with an aluminum tree tag. This arrangement facilitated rerandomization of lures between traps while minimizing the potential for cross-contamination of traps. Lures were replaced at 2-wk intervals as in the previous study.

In 1998, traps were deployed in Franklin, Wake, Chatham, Randolph, and Henderson Counties. Traps were deployed the first week of June and were monitored weekly through the first week of September. Lures were rerandomized between traps each time they were checked. Traps were deployed in Franklin, Wake, Chatham, and Randolph in 1999 and were checked and rerandomized weekly from the first week of June through the second week of September. In 2000, traps were deployed only in Wake and Wayne Counties. Traps were deployed in Wake County in the last week of March and in Wayne in the second week of June and were checked and rerandomized weekly.

A ratio of *E* and Z strain captures was calculated for each observation, and the ratios were converted by the $(x + 0.5)^{1/2}$ transformation before being subjected to analysis of variance (ANOVA) through the GLM procedure (SAS Institute 2000), with year and county as main effects and year by county as an interaction of interest. County means were separated through least significant difference (LSD; $\alpha = 0.05$). In addition, the Fisher exact test was used to assess ratios of captures to each lure as measured by moth count totals between studies (1985–1988 and 1998–2000) and between apparent flights within counties; these analyses were conducted with actual moth counts.

Results

A total of 3,929 male European corn borer moths were captured over the course of this study, with 1,415 (36.0%) taken in *E*-baited traps, 2,479 (63.1%) taken in Z-baited traps, and 35 (0.9%) taken in unbaited traps. Both *E*-baited and Z-baited traps captured moths in all counties in all years, but the relative proportions of *E* and Z captures differed significantly among counties ($F = 17.75$; df = 5,319; $P < 0.001$; Table 1). The proportion of captures in *E*-baited traps in Henderson County, in the far west of North Carolina, was significantly lower than in all other counties. The relative proportion of *E* captures in Randolph and Chatham counties did not differ from each other but did from all others; the ratios in these counties were intermediate to those in Henderson County and the eastern group of counties (Wake, Wayne, and Franklin). No differences were detected between the three eastern

Table 1. Combined total captures of *E*- and *Z*-responsive European corn borer moths in six North Carolina counties for the years 1998–2000

County	No. trap replicates	No. years	*E* strain captures	Z strain captures	Ratio *E:Z*[a]
Chatham	6	2	88	378	0.23a
Franklin	6	2	378	687	0.55b
Henderson	3	1	2	87	0.02c
Randolph	6	2	172	961	0.18a
Wake[b]	9	3	676	340	1.98b
Wayne	3	1	99	26	3.80b

E: Z ratios are expressed as the no. of moths captured in *E*-baited traps for each moth captured in Z-baited traps for each county (x*E:* 1Z).

[a] Means followed by the same letter not significantly different at $P = 0.05$ (LSD).

[b] Includes adjacent areas of Harnett County.

counties. For those counties with multiple years of observation, no difference in ratio was observed between years ($F = 1.01$; df = 3,319; $P > 0.05$).

In all but two instances, captures in unbaited traps were of single individuals; in those two exceptions, a female European corn borer moth was retrieved from the trap along with the captured males. The greatest incidence of captures in unbaited traps occurred in 1998 in Wake county, when 11 of 497 moths were removed from such traps. These data suggest that traps were not cross-contaminated when lures were rotated among traps within a field.

Significant differences were detected in relative captures between the second and third flights each year (Table 2). The general trend was toward increased captures in Z-baited traps in the third flight; indeed, in each instance where the Fisher exact test ($P = 0.05$) detected a significant difference, it was in this direction.

Fisher exact test analyses ($P = 0.05$) of proportional captures between the 1980s study (Sorenson et al. 1992) and this study found a significantly higher proportion of *E* captures in Franklin County but significantly lower *E* capture rates in Chatham and Wayne Counties in the later study (Table 3). The relative proportions of captures by the two lures were unchanged between the two studies in Wake, Randolph, and Henderson Counties.

Table 2. Comparison of second flight (June–July) and third flight (late July–Aug.) European corn borer captures in pheromone traps in six North Carolina counties

County	Year	Second flight, *E:Z*	Third flight, *E:Z*
Chatham	1998	0.27 (25:92)	0.24 (24:99)
Chatham	1999	0.22 (31:144)	0.26 (11:43)
Franklin	1998	3.23 (278:86)	0.56 (20:36)[a]
Franklin	1999	1.58 (38:24)	0.40 (31:78)[a]
Henderson	1998	0.05 (2:44)	0.00 (0:43)
Randolph	1998	0.33 (102:306)	0.16 (60:368)[a]
Randolph	1999	0.05 (11:232)	0.10 (9:90)
Wake	1998	5.00 (255:51)	1.74 (108:62)[a]
Wake	1999	3.51 (200:57)	1.31 (110:84)[a]
Wake	2000	8.00 (8:1)	0.63 (12:19)[a]
Wayne	2000	6.50 (78:12)	1.50 (21:14)[a]

E: Z ratios are expressed as the no. of moths captured in *E*-baited traps for each moth captured in Z-baited traps for each county (x*E:* 1Z).

[a] Early summer ratios and late summer ratios significantly differ at $P = 0.05$ (Fisher exact test).

Table 3. Combined total captures of *E*- and *Z*-responsive European corn borers in six North Carolina counties, 1977–1979 (Kennedy and Anderson 1980), 1985–1988 (Sorenson et al. 1992), and 1998–2000

County	Ratio *E:Z*, 1977–1979	Ratio *E:Z*, 1985–1988	Ratio *E:Z*, 1998–2000
Chatham	—	0.31 (28:59)	0.23 (88:378)[a]
Franklin	—	0.65 (34:52)	1.22 (378:309)[a]
Henderson	—	0.03 (6:183)	0.02 (2:87)
Randolph	—	0.23 (21:92)	0.18 (172:961)
Wake	0.35 (142:404)	1.90 (601:317)	1.98 (676:340)
Wayne	—	18.20 (91:5)	3.81 (99:26)[a]

E:Z ratios are expressed as the no. of moths captured in *E*-baited traps for each moth captured in Z-baited traps for each county (x*E:* 1Z).

[a] 1985–1988 and 1998–2000 ratios significantly differ at $P = 0.05$ (Fisher exact test).

Discussion

The distribution of pheromone races of the European corn borer, as determined by pheromone trap captures, seems to have changed little over ≈10 yr between the previous study, which spanned 1985–1988 (Sorenson et al. 1992), and this study, which spanned 1998–2000. *E* race captures seem to continue to dominate in the coastal plain of North Carolina, whereas the European corn borer population in the far western regions of the state continues to consist essentially of only *Z* race moths. In the ≈10 yr between the studies of Kennedy and Anderson (1980) and Sorenson et al. (1992), it seemed that the eastern *E* populations had expanded into an area (Wake County) previously dominated by a *Z* population. Why this western expansion of the *E* race did not continue over the subsequent 10 yr is unknown. Extensive woodlands and relatively limited agriculture characterize the region between Wake County and Chatham and Randolph Counties, possibly forming a physical barrier between the eastern and western populations. Moreover, the agriculture that does exist in this area is relatively low in diversity; the only agricultural host of the European corn borer that is widely planted in this region is maize, and the land area dedicated to maize has declined by ≈30% across the region and in the state between 1988 and 1998 (National Agricultural Statistics Service 1994–2001).

Between 1985 and 2000, urbanization removed ≈14,200 ha of cropland from production in Wake County (Fig. 2); this represents a 68% decline (North Carolina Department of Agriculture 1986–2003). Over this same period, land devoted to maize production declined by 95%, from >4,000 to ≈200 ha. Large blocks of suitable European corn borer host have therefore become very rare in much of Wake County; this loss of suitable habitat may be limiting the size of local European corn borer populations. Such land use

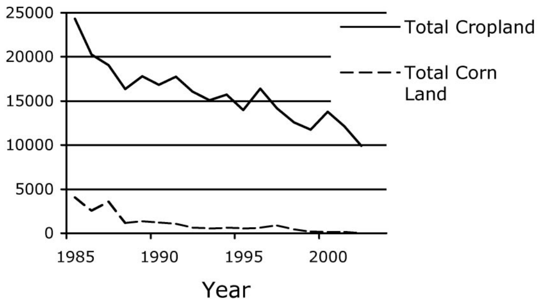

Fig. 2. Total cropland and maize cropland in Wake County, NC, 1985–2002.

changes may contribute to the relative stasis in racial composition of European corn borer populations in Wake County over the intervening years.

The situation in the zone of extensive intergradations between the two forms, represented by the eastern Piedmont counties of Franklin, Chatham, and Wake, and the western (inner) coastal plain county of Wayne, suggests that a dynamic relationship continues to exist between the two forms in areas of sympatry. In the more eastern Piedmont county (Franklin), the proportion of *E* captures increased over that observed in the previous study, whereas in Wayne, located further to the east and ≈75 k to the south, the proportion of *E* captures declined since it was last measured. In the previous study, significant variation between years was occasionally detected in this zone of overlap. *E* and *Z* race populations of the European corn borer exhibit clear differences in performance on hosts other than maize (Straub et al. 1986, Eckenrode and Webb 1989) and in pheromone calling behavior (Webster and Cardé 1982). Variations in proportional captures between years could well be caused by vagaries in the performance of one race compared with the other under variable environmental conditions and host plant availabilities.

As in the previous study, significant differences in the composition of the second and third flights were observed in Wake, Franklin, Randolph, and Wayne Counties. In all these cases, the prevalence of *E* race moths declined in the third flight compared with the second. Why this rather consistent decline in relative abundance of the *E* strain occurs is open to speculation, but it may be caused by host–plant performance differences (i.e., one strain performing better in maize) or other differences in biology. Recent work by Malausa et al. (2005) in France documented assortative mating between sympatric populations of the *E*, or hop-mugwort (*Humulus lupulus* L. and *Artemisia vulgaris* L.), and *Z*, or maize, strains. In their work, they reported very high levels of host fidelity in each strain and virtually absolute reproductive isolation. Host fidelity in North Carolina may not be as strong, at least in the *E* strain, because large numbers of European corn borer larvae have routinely been collected from maize (and other crops, including cotton and potato) in far eastern parts of the state where populations are essentially all *E* strain as measured by pheromone response (Sorenson et al. 1992, 1993). However, the same forces driving assortative mating in France could be at work in North Carolina, and could account, at least in part, for the seasonal variations we observed.

The taxonomic relationship between the strains has been the subject of debate for quite sometime. While *E* and Z strain moths do interbreed, they probably do not do so freely (Cardé et al. 1978), and our data seem to support this contention. However, the genetic control of the pheromone races seems to be relatively simple. Female pheromone production seems to be regulated by a single autosomal gene, whereas male pheromone response is mediated by another, sex-linked, gene, and there is little linkage between the

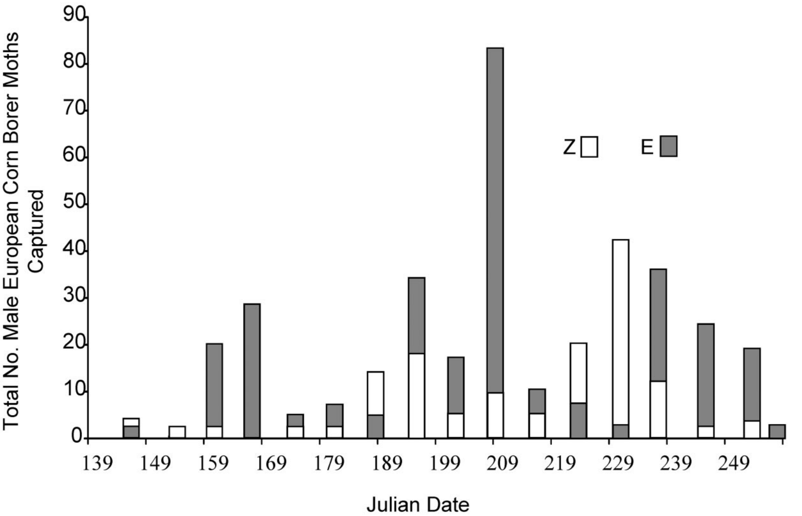

Fig. 3. Captures of male European corn borers in pheromone traps in Franklin County, NC, 1998.

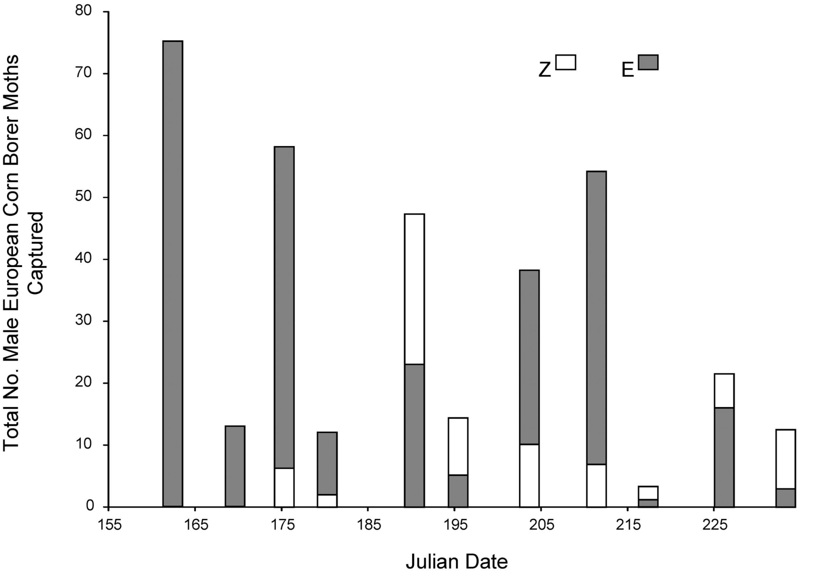

Fig. 4. Captures of male European corn borers in pheromone traps in Wake County, NC, 1999.

two (Roelofs et al. 1987, Löfstedt et al. 1989, Glover et al. 1990). Further complicating the situation, male *E* race moths seem to be less specific in their response to various pheromone blends than Z race males (Linn et al. 1997); in areas of sympatry, there may be some gene flow between the races, largely because of matings between *E* race males and Z race females (Glover et al. 1991). The pheromone races of the European corn borer seem to have emerged relatively recently (Marçon et al. 1999, Willett and Harrison 1999), probably in response to a host shift to maize when it was introduced to Europe ≈500 yr ago (Thomas et al. 2003).

In this study and the previous study (Sorenson et al. 1992), we detected patterns of captures suggesting that the *E* race and *Z* race populations respond more or less independently to some unidentified environmental cues. In some areas included in our study, notably those areas where substantial populations of both races seem to occur together, there are large fluctuations from year to year in the proportionality of captures; in these same areas, we often detected differences in the proportionality between flights within years. Additionally, we detected temporal asynchrony between the races within a growing season in several instances, with *Z* race peak flights as measured by pheromone trap captures occurring between *E* race flights (Figs. 3 and 4). Thomas et al. (2003) reported asynchrony in populations in northern France. In that region, the *E* race and *Z* race apparently exhibit very high host fidelity to hop-mugwort and maize, respectively, and *E* race moths emerge on average ≈10 d before *Z* race moths. Our data suggest a more complicated situation; while we do observe asynchrony in flights, the temporal pattern of this asynchrony varies from year to year and site to site.

Our understanding of the relationships between these two forms in North Carolina, and in particular in the zone of overlap, would be clarified by additional studies, particularly an examination of pheromone production by females, detailed studies of host affiliation, and postdiapause development in the two races.

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References Cited

Cardé, R. T., J. Kochansky, J. F. Stimmel, A. G. Wheeler, Jr., and W. L. Roelofs. 1975. Sex pheromone of the European corn borer (*Ostrinia nublialis*): cis- and trans- responding males in Pennsylvania. Environ. Entomol. 4: 413–414.

Cardé, 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.

Eckenrode, C. J., and D. R. Webb. 1989. Establishment of various European corn borer (Lepidoptera: Pyralidae) races on selected cultivars of snap beans. J. Econ. Entomol. 82: 1169–1173.

Glover, T. J., M. Campbell, P. Robbins, and W. L. Roelofs. 1990. Sex-linked control of pheromone behavioral responses in European corn borer moths, *Ostrinia nubilalis*, confirmed with TPI marker gene. Arch. Insect Biochem. Physiol. 15: 67–78.

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. Environ. Entomol. 20: 1356–1362.

Kennedy, G. G., and T. E. Anderson. 1980. European corn borer trapping in North Carolina with various sex pheromone component blends. J. Econ. Entomol. 73: 642–646.

Klun, J. A., and S. Maini. 1979. Genetic basis of an insect chemical communication system: the European corn borer. Environ. Entomol. 8: 423–426.

Kochansky, J., R. T. Cardé, J. Liebherr, and W. L. Roelofs. 1975. Sex pheromone of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), in New York. J. Chem. Ecol. 1: 225–231.

Linn, C. E., Jr., 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.* Physiol. Entomol. 22: 212–223.

Löfstedt, C., B. S. Hansson, W. L. Roelofs, and B. O. Bengtsson. 1989. No linkage between genes controlling female pheromone production and male pheromone response in the European corn borer, *Ostrinia nubilalis* Huebner (Lepidoptera: Pyralidae). Genetics. 123: 553–556.

Malausa, T., M.-T. Bethenod, A. Bontemps, D. Bourguet, J. -M. Cornuet, and S. Ponsard. 2005. Assortative mating in sympatric host races of the European corn borer. Science. 308: 258–260.

Marçon, P.C.R.G., D. B. Taylor, C. E. Mason, R. L. Hellmich, and B. D. Siegfried. 1999. Genetic similarity among pheromone and voltinism races of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). Insect Molec. Biol. 8: 213–221.

National Agricultural Statistics Service. 1994–2001. Agricultural Statistics. http://www.usda.gov/nass/pubs/agstats.htm.

North Carolina Department of Agriculture. 1986–2003. North Carolina Agricultural Statistics. North Carolina Department of Agriculture and Consumer Services, Raleigh, NC.

Roelofs, W. L., T. Glover, X. H. Tang, I. Sreng, P. Robbins, C. Eckenrode, C. Löfstedt, B. S. Hansson, and B. O. Bengtsson. 1987. Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. Proc. Nat. Acad. Sci. U.S.A. 84: 7585–7589.

SAS Institute. 2000. SAS user's guide: statistics. SAS Institute, Cary, NC.

Sorenson, C. E., G. G. Kennedy, J. W. Van Duyn, J. R. Bradley, Jr., and J. F. Walgenbach. 1992. Geographical variation in pheromone response of the European corn borer, *Ostrinia nubilalis,* in North Carolina. Entomol. Exp. Appl. 64: 177–185.

Sorenson, C. E., G. G. Kennedy, J. W. Van Duyn, and J. R. Bradley, Jr. 1993. Distribution of second generation European corn borer, Ostrinia nubilalis, egg masses in field corn and relationship to subsequent tunneling damage. Entomol. Exp. Appl. 68: 15–23.

Straub, R. W., R. W. Weires, and C. J. Eckenrode. 1986. Damage of apple cultivars by races of European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 79: 359–363.

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* Hübner. I. Sex pheromone, moth emergence timing, and parasitism. Evolution. 57: 261–273.

Webster, R. P., and R. T. Cardé. 1982. Influence of relative humidity on calling behaviour of the female European corn borer moth (*Ostrinia nubilalis*). Entomol. Exp. Appl. 1982, 32: 181–185.

Willett, C. S., and R. G. Harrison. 1999. Insights into genome differentiation: Pheromone-binding protein variation and population history in the European corn borer (*Ostrinia nubilalis*). Genetics. 153: 1743–1751.

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