Effectiveness of Four Blends of European Corn Borer (Lepidoptera: Pyralidae) Sex Pheromone Isomers at Three Locations in South Carolina

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ABSTRACT A field study was conducted at Florence, Newberry, and Clemson, South Carolina, to determine the relative attractiveness of four isomeric blends of 11-tetradecenyl acetate to male moths of the European corn borer (ECB), Ostrinia nubilalis (Hubner). The E and Z isomers were formulated in E:Z ratios of 99:1, 97:3, 65:35, and 3:97. Trap capture data indicated that the E pheromone strain of the ECB was predominant at Florence and the Z strain was predominant at Clemson. Both strains appeared to occur sympatrically at Newberry. Pheromone analysis of 46 ECB female moths at Florence indicated that 43 were the E strain and 3 were hybrids. The 99E:1Z pheromone blend captured over four times as many male moths as the 97E:3Z blend at Florence. The 65E:35Z blend captured 5%, 11%, and 12% of the moths at Florence, Newberry, and Clemson, respectively, indicating the possible presence of hybrid moths. The superior performance of the 99E:1Z blend of 11-tetradecenyl acetate should enhance the development of a more effective lure for the E strain of the ECB.

KEY WORDS European corn borer, Ostrinia nubilalis, Lepidoptera, Pyralidae, pheromones, pheromone trapping, (E)-11-tetradecenyl acetate, (Z)-11-tetradecenyl acetate.

The identification of (Z)-11-tetradecenyl acetate as a sex stimulant of the European corn borer (ECB), Ostrinia nubilalis (Hubner) (Lepidoptera: Pyralidae), by Klun and Brindley (1970) led to the use of pheromone traps for monitoring this important pest of corn, cotton, sorghum, and vegetable crops in the eastern United States. Male moths in Iowa responded to this pheromone but were inhibited by the
isomer (Klun and Robinson 1971). Klun et al. (1973) reported that optimum attractiveness occurred in Iowa when 4% E isomer was added to the Z isomer, and that geometrically pure preparations of the Z isomer were relatively unattractive. Kochansky et al. (1975) observed that maximal attractiveness occurred in New York when 2% of the Z isomer was added to the E isomer. Roelofs et al. (1985) analyzed pheromone blends from glands of female ECB and reported the existence of three distinct populations of ECB in New York based on sex pheromones and voltinism. These populations were a bivoltine biotype utilizing the Z isomer as the primary pheromone component, a univoltine Z biotype, and a bivoltine E biotype. Ratios of E:Z were not constant among biotypes, with the percentage of E isomer ranging from approximately 0–27 at a location where the Z strain predominated and the percentage of Z isomer ranging from approximately 0–24 at a location where the E strain predominated. Several moths which produced approximately 59–78% E isomer were presumed to be hybrids. Glover et al. (1987) analyzed behavioral responses of male ECB from New York to 11-tetradecenyl acetate and found that univoltine and bivoltine Z biotypes exhibited the greatest response to the 3% E isomer compared with the 0, 0.5, and 1% E isomers, whereas the bivoltine E strain produced and responded better to the 1% Z isomer compared with the 0, 0.5 and 3% Z isomers. Differences among responses for each bivoltine biotype were nonsignificant for the 0.5, 1, and 3% isomers, but were significantly greater than for the 0% isomers. For the univoltine Z biotype, response to the 1 and 3% E isomers was significantly greater than for the 0 and 0.5% E isomers.

Studies conducted in the southeastern United States document the sympatric occurrence of E and Z strains. Kennedy and Anderson (1980) reported that E and Z strains occurred sympatrically in North Carolina. They captured significantly more moths in the 4E:96Z traps than in the 96E:4Z traps, and stated that the occurrence of significant catches with 50E:50Z and 65E:35Z blends may indicate the existence of F1 hybrids. DuRant et al. (1986) and DuRant and Manley (1987) reported the sympatric occurrence of both the E and Z strains of ECB in South Carolina, with the E strain predominating in eastern South Carolina and the Z strain predominating in western South Carolina. DuRant and Manley (1987) compared Trece New York (E) lures with Trece Iowa (Z) lures in several locations in South Carolina. The E lures captured 91.8%, 10.9%, and 1.1% of the ECB moths at Florence in northeastern South Carolina, Newberry in central South Carolina, and Clemson in northwestern South Carolina, respectively. Although both lures captured significant numbers of ECB males in central South Carolina, captures for the Z blend at Florence and for the E blend at Clemson did not differ significantly from captures for the unbaited traps.

In order to successfully monitor populations of ECB, both traps and lures must be efficient. Kennedy and Anderson (1980) concluded that sticky traps were inadequate for timing insecticide treatments in North Carolina, but Fletcher-Howell et al. (1983) reported that pheromone traps provided reliable monitoring of ECB. They attributed low second-flight catches to improper trap placement. Webster et al. (1986) and DuRant et al. (1986)
reported that *Heliothis* Sentry cone traps captured approximately seven times as many male ECB as Pherocon 1C wing traps. DuRant et al. (1986) compared commercial lures (Albany, Zoecon [Trece]) with lures formulated by R. T. Cardé (Department of Entomology, University of Massachusetts, Amherst). The Zoecon E lure, but not the Albany E lure, captured significantly more male ECB than the Cardé E lure (97E:3Z) in eastern South Carolina. A preliminary study which we conducted in eastern South Carolina during 1992 indicated that lures containing 99E:1Z were significantly more efficient than 95E:5Z lures and Trece E lures. Lures containing 99E:1Z captured approximately 10 times as many male ECB as either of the latter two lures.

The primary objective of the present study was to evaluate the relative attractiveness of four blends of (E)- and (Z)-11-tetradecenyl acetate to male ECB in three locations in South Carolina that appear to differ in predominance of the pheromone strains of the ECB. An additional objective of this study and studies conducted during 1991 and 1992 (J.A.D., unpublished data) was to determine if the relative abundance of the pheromone strains had changed since 1986 (DuRant and Manley 1987).

**Materials and Methods**

**Pheromone gland analysis.** Diapausing ECB larvae were collected from sorghum plants at Florence from 28 January - 3 February, 1993, and shipped to C.E.M. at the University of Delaware for sex pheromone gland analysis. Each larva was held in a 28 ml plastic food service cup containing a cotton dental wick saturated with water to provide a source of moisture. Larvae were maintained in a reverse photoperiod (dark cycle initiated at 1000 hours) under nondiapause conditions at 25 ± 2°C, 16:8 (L:D) photoperiod, and a relative humidity of 50-80%. Pupae were monitored daily until adult eclosion and pheromone gland removal.

Following methods similar to Roelofs et al. (1985), pheromone glands from adult females were excised just anterior of the ring gland. Glands were excised at the sixth hour of scotophase on the second day after eclosion (moths 24-48 h old). Glands were placed with a dissecting needle into 6 μl of heptane containing 1.8 ng cis-7-tetradecenyl acetate as an internal standard in a point-tipped autosampler vial for at least 30 min and injected in a Varian 3500 gas chromatograph (Varian Associates, Sunnyvale, California) after being evaporated to approximately 3 μl. Injections of 3 μl were made using a sandwich technique where a 0.5 μl air gap was placed between the solvent plug and sample plug in a 10 μl syringe, thus preventing contact and sample contamination by the solvent plug. The gas chromatograph was equipped with a Varian 1077 split/splitless injector fitted with a 4 mm ID open-top glass uniliner (Restek Corporation, Bellefonte, Pennsylvania) containing glass wool, a fused silica capillary column 60 m by 0.25 mm with 0.25 μl Stabilwax® film thickness (Restek Corporation), a 5 m by 0.25 mm fused silica guard column, and a flame ionization detector. The gas chromatograph was programmed as follows: injector at 200°C, splitless for 2.5 min, then set to split for the remainder of the run (split ratio 50:1 set at
detector at 250°C, attenuation set at 32-11; column oven programmed at 60°C, hold 1.0 min, heat from 60-240°C at 8°C/min, hold at 240°C to end of the run; and total run time was 32 min. Nitrogen was used as carrier gas at a flow rate of 19 cm/sec and as makeup gas. Under these conditions, the internal standard and two pheromone isomers eluted at approximately 28 min with each of the three peaks being separated by 0.2-0.4 min which allowed for distinct separation and detection of these compounds.

Female moths for which pheromone glands were excised and analyzed by gas chromatography were categorized by pheromone strain based on the percentage ratio of the two pheromone isomers. The percentages were determined by comparison of peak heights of the isomers at the appropriate retention times on the chromatogram. Samples with the peak height consisting of 85% or more of the E isomer compared with the Z isomer were classified as E strain, those with 35-80% E isomer were classified as hybrids, and those with 15% or less were classified as Z strain. Samples were not classified if there was insufficient quantity of pheromone as indicated by the lack of gas chromatography peaks at the appropriate retention times.

Preparation of lures. (E)- and (Z)-11-tetradecenyl acetate were purchased from Sigma Chemical Company, St. Louis, Missouri. Stock solutions of each isomer were prepared by adding 0.4 ml of pheromone to 3.6 ml of heptane for lures used during April and May, and by adding 0.1 ml of pheromone to 9.9 ml of heptane for lures used during the remainder of the season. Four pheromone blends, 99E:1Z, 97E:3Z, 65E:35Z, and 3E:97Z, were prepared by combining the appropriate proportions of the stock solutions with additional heptane to obtain a concentration of 100 μg pure pheromone per 12.5 μl total solution. Lures were prepared by pipetting 12.5 μl of the appropriate blend onto a silicon rubber septum (rubber stopper, red, sleeve type, 5 by 9 mm, Arthur H. Thomas Company, Swedesboro, New Jersey). Septa were placed under a fume hood for at least 1 h to evaporate the excess heptane and subsequently held in sealed glass jars in a freezer until needed.

Analysis of pheromone blends. Samples of the stock solutions of the four pheromone blends were shipped to C.E.M. at the University of Delaware and H.W.F. at Clemson University for determination of the percentage composition of each isomer.

At the University of Delaware, each stock solution was serially diluted (1:1,000) with heptane to obtain a solution containing approximately 10 ng/μl of the combined weight of the two isomers. Injection of 3 μl of this solution was made in the gas chromatograph using the same procedure as for the pheromone gland analysis except that a 30 m by 0.25 mm fused silica capillary column with 0.5 μm Stabilwax® film thickness was used and the total time for the run was reduced to 26 min. Under these conditions, the internal standard and two pheromone isomers eluted at approximately 24 min with each of the three peaks being separated by 0.2-0.3 min. Two runs were made of each of the blends tested. Detector response was recorded with a computer using a GC Star Workstation program (Varian Associates) which computed the areas of the peaks. Area for the (E)- and (Z)-11-tetradecenyl acetate peaks on the chromatogram was used to determine the percentage of each isomer in the blends.
At Clemson University, each stock solution was diluted 1:40 with heptane. These dilutions were injected (3 μl) without splitting into a gas chromatograph (Varian 3400 equipped with a Varian 8100 autosampler; Varian Associates). A 60 m by 0.25 mm fused silica capillary DB-wax column with 0.25 μm film thickness (J & W Scientific, Folsom, California) was used with the following conditions to improve separation, injector at 250°C, detector at 300°C, oven programmed for a 0.45 min hold at 90°C followed by an increase at 25°C/min to 220°C where temperature was held for 23 min. The head pressure of the columns was 703 g/cm². Peaks were detected using flame ionization. A Varian DS601 data system (Varian Associates) was used to integrate peak areas as a percentage of the area in the peaks for the two isomers. Dilutions containing 100% of either isomer were 99.9% pure with no cross-contamination between the isomers.

**Field study.** Lures were positioned in the center of the entrance (lower opening) of Albany Heliothis Scentry traps (United Agri Products, Fresno, California) using a taut wire. Traps were attached to metal conduit pipes so that the entrance was 1 m above the soil surface, and were arranged in a linear, randomized complete block design with three replications of each treatment (pheromone blend) adjacent to field boundaries. The unbaited traps contained lures impregnated with 12.5 μl pure heptane. A fifth treatment, the Trece E (NY) lure (rubber stoppers, red, sleeve type, 5 by 9 mm, Pest Management Supply, Inc., Amherst, Massachusetts), was evaluated at Florence beginning on 10 June. Traps and replications were at least 30 m apart. All traps within each replication were monitored and rerandomized weekly (6-8 d intervals), at which time lures were replaced. ECB males were removed weekly and identification was confirmed when necessary by examination of genitalia (Mutuura and Munroe 1970).

Locations (dates in 1993) monitored were Florence (1 April - 2 September), Newberry (30 March - 1 September), and Clemson (25 March - 2 September). At Florence, all traps initially were located in the edge of a wheat field. Fields in this vicinity had contained corn and cotton the previous season (1992). On 3 June all traps were moved into the edge of a corn field which was adjacent to the wheat. Although the corn field was irrigated by a center pivot system, all trap locations were not within the irrigated area. Three, four, and four of six traps in replicates one, two, and three, respectively, were located outside the irrigated area. At Newberry, all traps initially were located in the edge of a corn field which had contained corn the previous season. On 3 June, all traps were moved into the edge of an irrigated corn field which had contained corn and soybeans the previous season. At Clemson, all traps were located in grassy areas adjacent to fallow fields. Corn was planted adjacent to the traps during late May. Trap captures were summed for the season and transformed to log (x + 1) for analyses, which were performed using the ANOVA-2 and RANGE programs of MSTAT-C. Means were separated using protected least significant difference (LSD) at the 5% level of probability (MSTAT Development Team 1988).
Results and Discussion

Analyses of pheromone blends indicated that the actual ratios of $E:Z$ were near the desired ratios (Table 1). Results of the analyses conducted at Clemson University differed from those of the analyses conducted at the University of Delaware, with the percentage of $E$ isomer being greater for the Clemson analyses than for the Delaware analyses for the 99$E$, 97$E$, and 65$E$ blends and less for the 3$E$ blend. These variations between the actual and desired ratios probably had little impact on the trap captures. Roelofs et al. (1985, 1987) found that ratios of $E:Z$ produced by ECB females varied widely for both the $E$ and $Z$ strains for mixed field populations, but exhibited very little variation in pure strains in laboratory cultures. Glover et al. (1987) reported that for bivoltine $E$ and $Z$ strains, differences in male responses were nonsignificant for pheromone blends containing from 0.5-3% of the minor isomer. The greatest variations from the desired ratios were observed for the Clemson University analyses of the pheromone blends used before 10 June. Since moth activity peaked after this date, any aberrations in trap capture data would have affected only a small percentage of the total number of moths captured.

Pheromone blend significantly influenced trap captures at all locations (Table 2). Moth captures at Florence ranged from 2.7-281.0 males/trap for the unbaited lure and 99$E$:1Z, respectively. The 99$E$:1Z blend was significantly superior to the remaining blends. The 97$E$:3Z blend was significantly superior to the 65$E$:35Z blend, which was significantly superior to the 3$E$:97Z blend and the unbaited lure. This trend was relatively consistent throughout the season, with the 99$E$:1Z blend producing the greatest trap captures for 15 of the 18 weeks during which moths were captured at Florence (Fig 1). Moth captures for the 97$E$:3Z lure and the Trece $E$ lure were nearly identical, with the 97$E$:3Z and Trece $E$ lures capturing 144 and 158 moths/traps, respectively (Fig. 2). Weekly variations in apparent relative efficacies between these two lures may have been influenced by trap location within each replication, since several trap locations for each replication were beyond the range of the irrigation system. Drought conditions during July and August 1993 severely affected the corn and other vegetation at these locations and the preference of the ECB moths for traps located within irrigated areas was obvious. However, these differences were minimized over the entire trapping period by the weekly rerandomization of the treatments.

Analysis of pheromone blends produced by ECB female moths reared from the larvae which had been collected from sorghum at Florence confirmed the presence of the $E$ strain. Of 56 pheromone glands analyzed by gas chromatography, 46 had sufficient peaks for the two pheromone isomers to be determined. The percentages of $E$ isomer for 43 classified as $E$ pheromone strain had a mean of 96.75%, standard deviation of 2.66%, and a range of 87.8-99.1%. The percentage of $E$ isomer for the three classified as hybrid had a mean of 69.4%, standard deviation of 5.82%, and a range of 65.0-76.0%. None were classified as $Z$ pheromone strain.
Table 1. Results of European corn borer pheromone blend analyses at Clemson University and the University of Delaware.

| Desired ratio (E:Z) | Clemson | | % E isomer | | Delaware | | % E isomer |
|--------------------|---------|-----------------|-----------|----------|----------|--------|
|                    | No.     | Mean % | Range | No.     | Mean % | Range |
| Lures used until 10 June (Florence, Newberry), 13 June (Clemson) | | | | | |
| 99:1               | 5 | 99.9 | NV<sup>a</sup> | 2 | 99.2 | 99.1 - 99.4 |
| 97:3               | 6 | 98.8 | 97.7 - 99.4 | 2 | 97.1 | 97.1 - 97.2 |
| 65:35              | 6 | 64.4 | 63.4 - 65.7 | 2 | 60.3 | 60.0 - 60.6 |
| 3:97               | 3 | 1.2 | 0.6 - 2.3 | 3 | 3.1 | 3.0 - 3.2 |
| Lures used after 10 June (Florence, Newberry), 13 June (Clemson) | | | | | |
| 99:1               | 3 | 99.2 | NV<sup>a</sup> | 2 | 98.7 | 98.6 - 98.8 |
| 97:3               | 3 | 97.1 | NV<sup>a</sup> | 2 | 96.8 | 96.7 - 96.9 |
| 65:35              | 4 | 67.0 | 66.2 - 68.5 | 2 | 66.3 | 66.2 - 66.4 |
| 3:97               | 1 | 3.0 | – | 2 | 3.9 | NV<sup>a</sup> |

<sup>a</sup> NV means no variation among samples.

The absence of the Z phenotype in the gland analysis and the very low number of males in the 3E:97Z traps indicate that the Z strain is not maintaining a reproducing population in the Florence area. The presence of the 6.5% hybrids in the gland analysis and the 4.9% males caught by the 65E:35Z traps suggest that immigration of Z strain adults is occurring occasionally in this population.

Moth captures at Newberry ranged from 0.3-36.3 males/trap for the unbaited lure and 3E:97Z, respectively (Table 2). The 3E:97Z blend was significantly superior to all remaining blends except 99E:1Z, and all blends significantly increased moth captures compared with the unbaited lure. This trend was relatively consistent throughout the season except for June, when the remaining three blends produced the greatest moth captures (Fig 3). The 65E:35Z traps captured 11.2% of the total males collected in baited traps suggesting that natural crossing of the two strains is occurring.

Moth captures at Clemson ranged from 0.7-84.0 males/trap for the unbaited lure and 3E:97Z, respectively (Table 2). The 3E:97Z blend was significantly superior to 65E:35Z which was significantly superior to the remaining blends. This trend was consistent throughout the season (Fig. 4). The 12.1% of total males captured in the baited traps for the 65E:35Z blend...
Table 2. European corn borer male moth captures in traps baited with four blends of (E)- and (Z)-11-tetradecenyl acetate at three locations in South Carolina during 1 April - 3 September, 1993.

<table>
<thead>
<tr>
<th>Blend (E:Z)</th>
<th>Mean number of males/trap (percentage of total)a,b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Florence</td>
</tr>
<tr>
<td>99.1</td>
<td>281.0a (77.0)</td>
</tr>
<tr>
<td>97:3</td>
<td>62.0b (17.0)</td>
</tr>
<tr>
<td>65:35</td>
<td>17.7c (4.9)</td>
</tr>
<tr>
<td>3:97</td>
<td>4.0d (1.1)</td>
</tr>
<tr>
<td>Unbaited</td>
<td>2.7d</td>
</tr>
</tbody>
</table>

a Means within a column followed by the same letter do not differ significantly (P < 0.05 by protected LSD) (MSTAT Development Team 1988). Data were transformed to log (x + 1) for analysis but original means are presented.
b Unbaited trap catches were excluded from percentage calculations.

appears high considering the relatively low percentage caught in the 99E:1Z and 97E:3Z traps combined and the 11.1% captured by the 65E:35Z traps at Newberry where relatively high proportions of both the E and Z strains were captured. This may be partially due to the response of the males of the E strain and the hybrids to a range of pheromone blends as discussed by Glover et al. (1991). Hybrid males tend to respond to intermediate blends (65, 50, and 35% E) and 3E:97Z, but not to 99E:1Z. The E strain males will also respond to intermediate blends in addition to the 99E:1Z blend. Perhaps another factor is that the low proportion of the E strain in the population may lead to most of its F1 progeny being hybrid. Also, there may be immigration of some E strain and hybrid individuals. Collectively, these factors might explain the relatively high percentage of males captured in the 65E:35Z traps at Clemson compared with Newberry. Pheromone gland analysis of females sampled simultaneously from the populations at the three locations used in this study is needed to provide additional information on the proportion of pheromone phenotypes and the degree of natural hybridization in South Carolina.

Comparison of the relative proportions of E and Z strains of the ECB for 1993 versus 1986 is impeded because only two lures, Trece E and Trece Z, were compared at each location during 1986 whereas in 1993 three E lures (99E:1Z, 97E:3Z, and Trece) were evaluated at Florence and two E lures (99E:1Z and 97E:3Z) were evaluated at Newberry and Clemson. Competition among these similar blends may have resulted in reduced capture of moths by each lure compared with the 3E:97Z lure. However, during 1991 and 1992 only Trece E and Trece Z lures were compared at all three locations (J. A. D.,
unpublished data). At Florence, the Trece E lure captured 91.8%, 95.9%, 92.9%, and 93.9% of the ECB moths during 1987, 1991, 1992, and 1993, respectively, indicating that no substantial change has occurred in the relative proportions of E and Z strains. At Newberry, the Trece E (97E:3Z in 1993) lure captured 10.9%, 22.6%, 23.0%, and 23.7% of the moths during these four years, indicating that the proportion of the E strain increased between 1986 and 1991, but since then has remained relatively stable. At Clemson, the Trece E (97E:3Z in 1993) lure captured 1.1%, 18.7%, 14.0% and 3.4% of the moths during 1987, 1991, 1992, and 1993, respectively, indicating that the proportion of the E strain increased between 1986 and 1991, followed by a gradual decline during the past two years.

The relatively low proportion of moths captured by the 65E:35Z lures at Newberry and the apparent relative stability of the ratios of the two pheromone strains at each location since 1986 indicate that genetic shift of sex pheromone blends has not occurred to any great extent. Glover et al.
Fig. 2. Weekly mean numbers of European corn borer moths captured by traps baited with a known blend of (E)- and (Z)-11-tetradecenyl acetate and a commercial lure at Florence, South Carolina during 1993.

(1991) reported that Z races of the ECB predominate over most of the ECB range in Europe and North America, whereas the E race is restricted to select locations in Switzerland, Italy, and eastern North America. They cited the unidirectional gene flow from the E populations into the univoltine Z populations in New York and the resulting apparent genetic isolation of the E race as a possible reason for the limited geographical range of the E strain.

The E pheromone strain of the ECB was predominant at Florence in northeastern South Carolina and the Z strain was predominant at Clemson in the northwestern portion of the state. Results for 1993 were consistent with earlier results (DuRant and Manley 1987) in that trap captures of the Z strain at Florence were not significantly greater than captures for the unbaited traps. Although analysis of pheromone glands of ECB females from Florence confirmed the presence of the E strain, but not the Z strain, at this location, the presence of the Z strain cannot be ruled out. A relatively low population of Z strain moths could have been missed due to the small number (46) of females analyzed. Both studies indicate that both strains occur sympatrically at Newberry, in central South Carolina. Possible hybrids
were detected at all three locations, indicating that both strains may occur at Florence and Clemson, possibly as a result of occasional immigration. This study confirmed the superior effectiveness of the 99E:1Z pheromone blend at Florence compared with the 97E:1Z blend and the Trece E lure. These results should enhance development of a more effective lure for the E strain of the ECB in South Carolina and at other locations where this strain occurs.
Fig. 4. Weekly mean numbers of European corn borer moths captured by traps baited with four blends of \((E)\)- and \((Z)\)-11-tetradecenyl acetate at Clemson, South Carolina during 1993.

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