Seasonal Responses of Corn Rootworm Beetles (Coleoptera: Chrysomelidae) to Non-pheromonal Attractants[1,2]

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ABSTRACT Western, *Diabrotica virgifera virgifera* LeConte, and northern, *D. barberi* Smith and Lawrence, corn rootworm beetles were captured in a cornfield at traps baited with volatile attractants during three portions of the season. In dent-stage maize (3-4 wk past flowering), traps baited with eugenol captured large numbers of *D. barberi*, whereas traps baited with p-methoxycinnamonitrile (p-MCN) or estragole captured large numbers of *D. v. virgifera*. The attactants were less effective in two earlier trials when maize was in the whorl or silking stage. Despite seasonal differences, baits containing p-MCN attracted significant numbers of *D. v. virgifera* during all three trials. Attractants were also tested simultaneously in plots of corn that were planted on one of three different dates. With *D. v. virgifera*, attractants were relatively less effective in plots of maize that were silking than in plots of whorl-stage or dough-stage maize; with *D. barberi*, attractants were effective only in plots of whorl-stage maize. Changes in phenology of maize appear to be at least partially responsible for seasonal variation in attractancy of non-pheromonal lures for rootworm beetles. This seasonal variation will affect the use of non-pheromonal attractants in management applications.

KEY WORDS *Diabrotica virgifera virgifera, Diabrotica barberi*, western corn rootworm, northern corn rootworm, phenology, attractants.

A variety of semiochemicals have shown promise as tools for managing populations of corn rootworms, *Diabrotica* spp. (Metcalf et al. 1987, Lance 1988). These chemicals include a group of non-pheromonal attractants that act either as plant kairomones or as highly active analogs of kairomones. For example, adults of the northern corn rootworm (NCR), *D. barberi* Smith and Lawrence, are polyphagous pollen feeders and are attracted by eugenol, a widely distributed floral volatile (Ladd et al. 1983). Western corn rootworm beetles (WCR), *D. virgifera virgifera* LeConte, are strongly attracted by specific combinations of volatiles that are known to occur in blossoms of *Cucurbita maxima* Duchesne, a favored source of pollen (Lampman and Metcalf 1987). *D. v. virgifera* also respond to several individual attractants, including indole (Andersen and Metcalf 1986), estragole (Lampman et al. 1987), and *p*-methoxcinnamonitrile (Metcalf and Lampman 1989).

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Responses of northern and western corn rootworm beetles to non-pheromonal attractants may vary during the course of the season (Andersen and Metcalf 1986; Lampman and Metcalf 1987; McGovern and Ladd 1988). Seasonal shifts in attractancy would affect the use of these compounds as components of monitoring systems or semiochemical-based baits. In this paper, we document seasonal variation in attractancy of several non-pheromonal volatiles and examine possible effects of phenology of maize (*Zea mays* L.) on attractancy.

Materials and Methods

Trapping Methods. Beetles were captured on yellow cardboard sticky traps (unbaited Pherocon AM traps). Traps were stapled to wooden lath stakes at ca. 1 m in height, and stakes for each trap line (replicate) were placed equidistant between two rows of maize with 7 m between adjacent traps. Volatile attractants were applied to cotton wicks (1 cm diameter × 3 cm dental rolls) that were held by a clip ca. 5 cm above the trap. On each collection date, wicks were replaced, and beetles were removed from traps and returned to the laboratory for determination of species and sex.

Seasonal Variation in Attractancy. Trapping studies were conducted in a single field of commercially grown maize during 15 - 18 July, 28 - 31 July and 24 - 27 August, 1987. Traps were baited with 50 mg of one of the following: indole, estragole, eugenol, or 4-methoxycinnamonitrile. A fifth and sixth treatment consisted of [indole + estragole] and [indole + 4-methoxycinnamonitrile], with 50 mg of either compound on separate wicks. The seventh (control) treatment was unbaited traps. For each trapping period, plots were set up in a randomized block design, with seven traps (one for each treatment) in each of five rows (7 m between rows). On each of the four days of each trial, we counted beetles on 12 plants per row of traps within the plot (see Tollefson 1986) and rated the phenology of a total of 20 plants on the scale of Ritchie and Hanway (1982).

Effects of Corn Phenology. Trapping was conducted 22 - 26 August, 1986, in a 45 × 180 m cornfield. The field was divided into five 9 × 180 m "blocks" that were, in turn, divided into three 9 × 60 m plots. In each plot, corn was planted in eight 56-m rows with 1 m between rows. Within each block, one plot was randomly assigned to each of three planting dates: 21 May, 19 June, and 11 July. Traps were placed on a line down the center of each plot and were baited with 200 mg of one of the following: indole, estragole, eugenol, all three (separate wicks), or no volatiles (control). On each trap date, we also counted all beetles on 2 plants at seven locations within each plot. On 26 August, we rated phenology of 10 arbitrarily selected plants within each plot.

Analysis. Data for each trap were pooled across days and analyzed to evaluate the influence of trapping period or planting date on the effectiveness of attractants. Initially, t-tests of logarithmically transformed data were used to compare capture on baited and unbaited traps for each attractant within each trapping period or planting date. Efficiency of each unbaited trap was computed by dividing numbers of beetles captured per day by the mean number of beetles that were counted visually on corn plants within the appropriate plot for that trapping period or planting date. Relative efficiency of each trap baited with volatiles was computed similarly by dividing capture on each baited trap by the number of beetles captured on the unbaited trap in the same plot (before dividing, one was added to total catch for each trap because some unbaited traps captured no beetles of a given species and sex). Data were then transformed logarithmically and analyzed separately for each attractant treatment using analysis of variance.

Results

Seasonal Variation in Attractancy. Plant phenology and beetle populations varied widely during the three trapping periods. Plants in the plot were classified as V12-V14 (tassel not yet emerged or just beginning to emerge on some plants), R1 (silk emerged and all green or just starting to turn brown), and R5 stage (most kernels dented) from 15 - 18 July, 28 - 31 July, and 24 - 27 August, respectively ($n = 80$). Numbers of both *D. barberi* and *D. v. virgifera* on plants in the plot increased about 5-fold between the first and second trapping periods and then declined somewhat as the season progressed (Table 1). The unbaited Pherocon AM traps captured 2.8 - 7.7 beetles per trap per day for each beetle counted per plant. Efficiency of unbaited traps did not vary measurably among trapping periods for *D. v. virgifera* ($F = 1.15$; d.f. = 2, 12; $p = 0.35$) but, for *D. barberi,* was significantly lower during the second period than the first (Table 1; $F = 4.96$; $P = 0.27$).

Table 1. Mean numbers of western and northern corn rootworms on maize plants and on unbaited Pherocon AM traps (± S.E.) at three periods of the season.

Trapping period	Beetles per plant (± S.E.; n = 240)	Beetles per trap per day (± S.E.) male	Beetles per trap per day (± S.E.) female	Trap efficiency*
		Northern Corn Rootworm Beetles		
15 - 18 July	0.1 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	7.1 ± 1.8 a
28 - 31 July	0.7 ± 0.1	1.6 ± 0.4	0.2 ± 0.2	2.8 ± 0.7 b
24 - 27 August	0.4 ± 0.0	1.8 ± 0.3	0.2 ± 0.1	5.3 ± 0.9 ab
		Western Corn Rootworm Beetles		
15 - 18 July	0.3 ± 0.0	1.5 ± 0.3	0.2 ± 0.1	5.9 ± 1.5 a
28 - 31 July	1.3 ± 0.1	8.0 ± 1.4	1.5 ± 0.4	7.5 ± 1.3 a
24 - 27 August	0.7 ± 0.1	2.3 ± 0.7	1.2 ± 0.1	4.8 ± 1.7 a

* Trap efficiency = (total beetles/trap/day)/(mean beetles/plant). Means that are not followed by the same letter are significantly different (Student-Neuman-Keuls test of log-transformed data).

Attractancy of the volatiles varied seasonally. For *D. barberi* and female *D. v. virgifera*, attractants were more effective during the 24 - 27 August period than in either of the two earlier trapping periods (Fig. 1). For *D. barberi,* seasonal variation in relative efficiency of eugenol-baited traps was significant (for males: $F = 6.04$; d.f. = 2, 12; $p = 0.015$, females: $F = 15.3$; $P < 0.001$). Indeed, eugenol failed to capture significantly more *D. barberi* than unbaited traps during the second trapping period (Fig. 1). With female *D. v. virgifera,* seasonal differences in trap

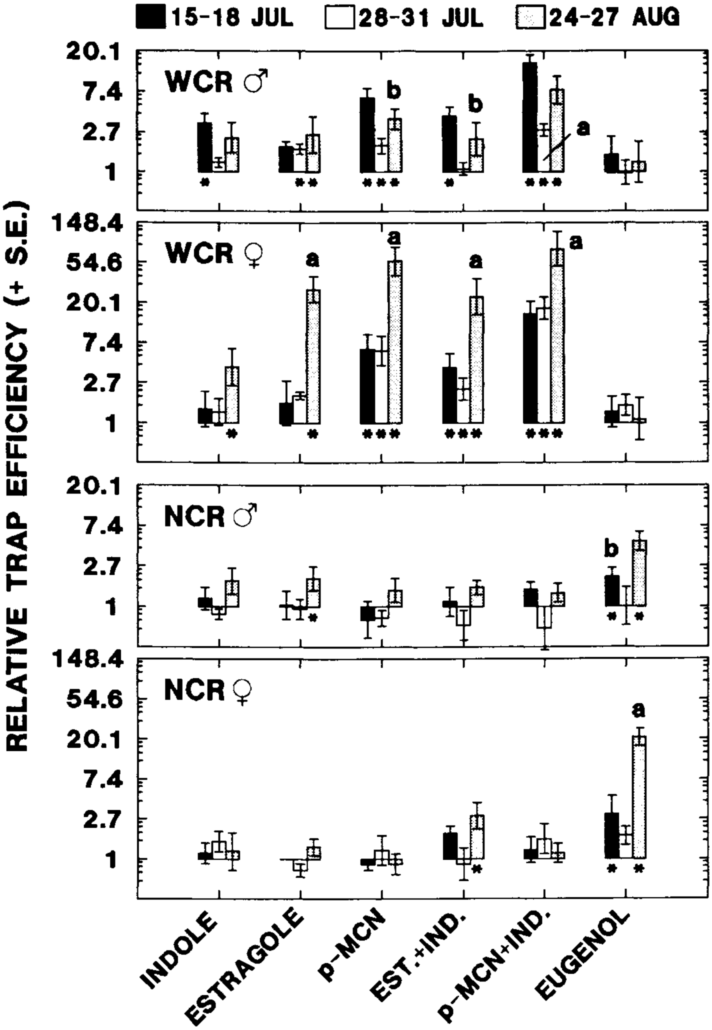

Fig. 1. Efficiency, relative to unbaited traps, with which sticky traps baited with various volatiles captured *D. v. virgifera* (WCR) and *D. barberi* (NCR) during 3 portions of the season (p-MCN = *p*-methoxycinnamonitrile). Asterisks(*) beneath bars indicate that capture on traps baited with the volatile was significantly greater than capture on unbaited traps (*t*-tests, α = 0.05). Bars under the letter "a" represent means that are significantly different from the other two means in the group, though the latter two means are significantly different from one another (Student-Neuman-Keuls' test, α = 0.05).

efficiency were significant for four of the six trap baits (estragole: $F = 17.8$; d.f. = 2, 12; $P < 0.001$, *p*-methoxycinnamonitrile: $F = 12.4$; $P = 0.001$, [estragole + indole]: $F = 10.9$; $p = 0.002$, [*p*-methoxycinnamonitrile + indole]: $F = 6.68$; $p = 0.011$). With male *D. v. virgifera,* effects of attractants on capture were greatest during the early period (15 - 18 July) and least pronounced during the second period (28 - 31 July); differences in relatively trap efficiency between the early and late periods were not significant (Fig. 1). Seasonal variation in relative capture of male *D. v. virgifera* was significant for traps baited with *p*-methoxycinnamonitrile ($F = 6.87$; d.f. = 2, 12; $P = 0.010$), [estragole + indole] ($F = 5.59$; $P = 0.019$), and [*p*-methoxycinnamonitrile + indole] ($F = 11.5$; $P = 0.002$). Despite seasonal differences, traps baited with *p*-methoxycinnamonitrile captured significantly more male and female *D. v. virgifera* than did unbaited traps during each of the 3 trapping periods (Fig. 1).

Effects of Plant Phenology. The use of three planting dates resulted in plots of maize in three distinct phenological categories during 22 - 26 August. Corn that was planted 21 May, 19 June, and 11 July were in R4 (dough stage; exposed silk thoroughly dry), R1 (green silks), and V8 (8-leaf stage), respectively. Numbers of rootworm beetles per plant were several times higher in flowering maize than in younger or older maize (Table 2). Planting date significantly affected the efficiency of unbaited traps for *D. barberi* (Table 2; $F = 28.8$; d.f. = 2, 12; $P < 0.001$) but not for D. V. virgifera ($F = 0.259$; P = 0.78).

Table 2. Mean numbers of western and northern corn rootworms on maize plants and on unbaited Pherocon AM traps (± S.E.) from 22 - 26 August, 1986, in plots of maize that were planted at one of three dates.

Corn phenology	Beetles per plant (± S.E.; n = 420)	Beetles per trap per day (± S.E.) male	Beetles per trap per day (± S.E.) female	Trap efficiency*
		Northern Corn Rootworm Beetles		
Pre-silking	1.0 ± 0.1	1.0 ± 0.2	0.6 ± 0.1	1.6 ± 0.3 b
Flowering	3.7 ± 0.3	1.5 ± 0.2	1.2 ± 0.2	0.7 ± 0.1 c
Dough stage	0.9 ± 0.1	3.3 ± 0.6	1.0 ± 0.3	4.6 ± 0.8 a
		Western Corn Rootworm Beetles		
Pre-silking	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	2.9 ± 1.8 a
Flowering	0.5 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	1.4 ± 0.2 a
Dough stage	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	3.2 ± 1.4 a

* Trap efficiency = (total beetles/trap/day)/(mean beetles/plant). Means that are not followed by the same letter are significantly different (Student-Neuman-Keuls test of log-transformed data).

For the more effective attractants, relative efficiency with which traps captured female beetles varied with the phenology of the surrounding maize (Fig. 2). With *D. barberi,* females responded more strongly to eugenol in whorl-stage maize than in plots of more mature plants (for eugenol alone: $F = 5.89$; d.f. = 2, 12; $P = 0.016$, estragole + indole + eugenol: $F = 4.98$; $p = 0.027$). Efficiency of estragole-baited traps for female *D. barberi* also varied with phenology ($F = 4.43$; $p = 0.036$), although estragole is not normally considered to be an attractant for northern corn rootworms (but see Lampman and Metcalf 1988). With *D. v. virgifera,* efficiency of traps baited with [estragole + indole + eugenol] was reduced in plots of flowering maize, but the effect was significant only for females (Fig. 2; $F = 7.23$; d.f. = 2, 12; $P = 0.009$, for males: $F = 3.64$; $P = 0.058$). In this study, attractants never produced a significant increase in capture within plots of flowering maize (Fig. 2).

Discussion

In these studies, responses of beetles to non-pheromonal attractants was lowest overall in plots of flowering maize. Similarly, Andersen and Metcalf (1986) noted that attraction of *D. v. virgifera* to sources of indole was reduced during the period of flowering. Also, Lance (unpublished data) found that eugenol lured few *D. barberi* to traps at the border of a cornfield during late July (around the period of flowering) but was a very effective attractant at the same site later in the season (also see McGovern and Ladd [1988]). Flowering maize produces an abundance of fresh silk and pollen, which are highly preferred foods for adult *D. barberi* and *D. v. virgifera* (Ludwig and Hill 1975, Lance and Fisher 1987). As suggested by Andersen and Metcalf (1986), beetles that have fed to repletion on preferred foods might show little or no response to food-related volatiles. Furthermore, volatiles from flowering corn are attractants for *Diabrotica* beetles (Prystupa et al. 1988) and perhaps compete with or mask odors given off at baited traps.

Although our data, in general, support the hypothesis that host phenology strongly influences responses of rootworm beetles to attractants, there are discrepancies in the results of our two studies. For female *D. v. virgifera*, relative efficiency of baited traps in the seasonal study (see Fig. 1) was similar in the whorl-stage (15 - 18 July) and flowering (28 - 31 July) trials but was much greater in the post-flowering (dent-stage) trial (24 - 27 August). In the planting-date study, however, responses of female *D. v. virgifera* to the multi-component attractant were greater in whorl-stage plots than in plots of flowering maize but were similar in plots of whorl- and post-flowering (dough-stage) maize (Fig. 2). Similarly, female *D. barberi* responded more strongly to eugenol in the post-flowering trial than in the two earlier trials of the seasonal study (Fig. 1), but, in the planting-date study, they showed significant response to eugenol only in plots of whorl-stage maize (Fig. 2).

Several factors could have caused the differences in relative responses of beetles to attractants in the two studies. Age of beetles, which could potentially affect their responses to volatiles, varied from repetition to repetition in the seasonal study but was presumably similar across plots of different phenology in the planting-date study. Also, the phenology of the post-flowering maize differed between the two studies. Plants were in the dent stage (R5) in the third repetition of the seasonal study, but were only in dough stage (R4) in the post-flowering plots of the planting-date study. In a subsequent season-long trapping study (G. R.

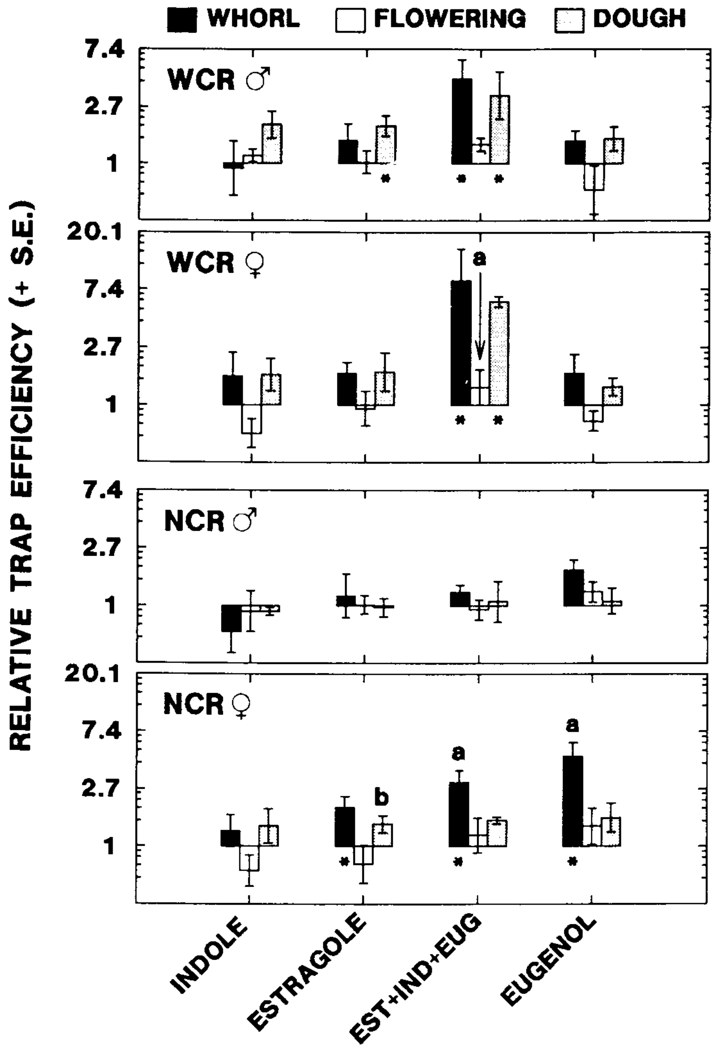

Fig. 2. Efficiency, relative to unbaited traps, with which sticky traps baited with various volatiles captured *D. v. virgifera* (WCR) and *D. barberi* (NCR) in plots of corn that were in three distinct phenological stages during the test. Asterisks (*) beneath bars indicate that capture on traps baited with the volatile was significantly greater than capture on unbaited traps (*t*-tests, $\alpha = 0.05$). Bars under the letter "a" represent means that are significantly different from the other two means in that group; bars under the letter "b" represent means that are not significantly different from the other two means in the group, though the latter two means are significantly different from one another (Student-Neuman-Keuls' test, $\alpha = 0.05$).

Sutter and D. R. L., unpublished data), reponses of *D. barberi* to cinnamyl alcohol (see Metcalf and Lampman [1989]) remained fairly low for an extended period and only began to increase when the surrounding corn reached the R4 stage. In the same study, relative efficiency of traps that were baited with an attractant for *D. v. virgifera* (*p*-methoxycinnamaldehyde; see Metcalf and Lampman [1989]) began to increase abruptly as soon as flowering ceased (R2 stage).

The relative results of our two studies also could have been influenced by the design of the plots in the planting-date study. Specifically, the proximity of flowering maize likely reduced relative trap efficiency to some degree in the plots of non-flowering maize. This effect was perhaps sufficient to cause the lack of significant attraction of female *D. v. virgifera* to estragole and indole in non-flowering plots. We have no reason to suspect, however, that the presence of flowering maize would have reduced the relative efficiency of traps in dough-stage maize to a greater degree than that of traps surrounded by whorl-stage maize.

Seasonal variation in the effectiveness of non-pheromonal attractants may have important implications for the use of these compounds in programs to manage rootworm populations. For example, reductions in attractancy during flowering could potentially limit the effectiveness of non-pheromonal volatiles as components of semiochemical-based toxic baits. Fortunately, these reductions may have relatively little influence on efficacy of baits, because the more potent attractants (e.g., *p*-methoxycinnamonitrile for *D. v. virgifera*) remain partially active throughout the season. Furthermore, the value of including volatile attractants in baits that are broadcast for rootworm control has not been conclusively demonstrated (see Lance and Sutter [1990]). In contrast, seasonal shifts in responses of beetles to attractants would profoundly influence the use of those volatiles in programs for monitoring populations of rootworm beetles. Previous research with unbaited sticky traps has indicated that the efficiency of capture for *D. v. virgifera* and (in agreement with our data) *D. barberi* tends to increase as maize progresses from flowering to maturity (Hein and Tollefson 1984, Matin et al. 1984). The addition of non-pheromonal attractants would probably improve the sensitivity and precision of trapping data but would also exaggerate seasonal shifts in trap efficiency. Intensive, season-long studies on specific combinations of attractants and traps will be required before capture data can be useful for making accurate management decisions.

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