

# Corn Phenology Influences *Diabrotica virgifera virgifera* Emigration and Visitation to Soybean in Laboratory Assays

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Environ. Entomol. 33(1): 35–44 (2004)

**ABSTRACT** We used two types of laboratory apparatus to test whether rotation-resistant and wild-type *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) responded differently to corn phenology. Beetles from Nebraska where injury to rotated corn has not been reported were defined as wild type, and beetles from Illinois where injury to rotated corn is common represented "rotation-resistant" populations. A two-chamber emigration arena assayed propensity of both populations to leave corn during and after anthesis. A side-arm olfactometer tested whether the relative attraction of beetles to soybean versus corn was influenced by corn phenology. Beetle origin did not influence departure from corn of varying phenology; both emigrated significantly less from young (shedding pollen) than old (no pollen) corn. Significantly more beetles entered olfactometer chambers with soybean than empty control chambers, but there was no difference in response between the two populations. Numbers of beetles entering chambers with soybean varied with the addition of young versus old corn. Replacing young with old corn approximately doubled the percentage of beetles not selecting corn. As corn aged, adult visitation of soybean increased significantly. We suggest this mechanism is sufficient to explain injury to rotated corn, when linked to a corn crop planted early and synchronously within a landscape limited to corn and soybean. This explanation based on preexisting behavioral plasticity should be given due consideration along with conceptual models of *D. v. virgifera* rotation resistance that imply genetic change.

**KEY WORDS** rotation, resistance, phenology, corn, soybean

IN MUCH OF THE NORTH CENTRAL region of the United States, annual rotation of corn, *Zea mays* L., and soybean, *Glycine max* (L.) Merr., has been the principal strategy for managing corn rootworms (Coleoptera: Chrysomelidae) (Levine and Oloumi-Sadeghi 1991). However, by the 1990s crop rotation had failed to protect against western corn rootworm, *Diabrotica virgifera virgifera* LeConte, larval injury to cornfields planted the previous year to soybean. This failure of crop rotation occurred in parts of the midwestern United States, primarily east central Illinois and northwestern Indiana (Levine et al. 2002). *Diabrotica v. virgifera* is univoltine; oviposition peaks in August (Hein and Tollefson 1985). The eggs undergo an obligatory diapause during the winter, hatch in late May through early June, and larvae feed on the roots of corn during June and July. Damage to annually rotated corn (first-year corn) occurs when adult female *D. v. virgifera* leave cornfields and oviposit in soybean fields (Spencer et al. 1999, Levine et al. 2002, O'Neal et al. 2002a). In areas where growers practice a strict corn-soybean rotation, larvae that emerge from these eggs the following year encounter corn whose roots they damage (O'Neal et al. 2001).

Before the 1990s, oviposition by *D. v. virgifera* was considered to occur primarily in cornfields (Levine

and Oloumi-Sadeghi 1991); thus, oviposition in soybean fields was attributed to the evolution of a beetle population distinct from the wild type. *D. v. virgifera* capable of injuring first-year corn rotated with soybean have been described as "behavioral resistance" (Onstad et al. 2001), "corn-soybean adapted" (Levine et al. 2002), "behavioral variant" (Sammons et al. 1997), or "strain" (O'Neal et al. 1999). All of these terms imply a genetic shift, like that associated with insecticide resistance. We will use the term "rotation resistance" to describe these populations having a history of damage to first-year corn. However, we suggest it is unclear whether genetic change is required for such behavior.

Based on olfactometer results, Sammons et al. (1997) suggested that injury to rotated corn was due to a behavioral biotype of *D. v. virgifera* that preferred soybean fields as oviposition sites. However, subsequent studies (Spencer et al. 1999) failed to confirm that adult *D. v. virgifera* from regions where injury to rotated corn is common select soybean over corn. The geographic pattern of the spreading injury to rotated corn was consistent with that expected for such a new *D. v. virgifera* biotype originating and dispersing from east central Illinois (Onstad et al. 1999). In addition, a simulation model (Onstad et al. 2001) based on

modification of a single gene locus was judged sufficient to explain rotation resistance; however, the behaviors altered by such a genetic change have not been specified, except that their expression requires a high degree of a corn-soybean rotation to be practiced within a landscape.

Spencer et al. (1999) investigated whether Illinois *D. v. virgifera* were attracted to soybean over corn in a flight chamber. Overall, a greater percentage of adult females were collected on corn than soybean, even though corn tassels and silks, both sources of volatile *D. v. virgifera* attractants (Metcalf 1986, Hammack 1997), were removed before testing. Spencer et al. (1999) concluded that rotation-resistant *D. v. virgifera* was not strongly attracted to soybean plants and that a preference for soybean was not necessary to explain movement into soybean. Rather, they suggested that reduced fidelity to corn may be sufficient to explain *D. v. virgifera* movement and oviposition in soybean fields (Spencer et al. 1999).

O'Neal et al. (2002a) observed a significant increase in feeding on soybean leaves when *D. v. virgifera* adults were presented with late vegetative versus early vegetative corn. The influence of corn phenology on adult feeding preference led O'Neal et al. (2002a) to support Spencer et al.'s (1999) proposal that reduced fidelity to corn was sufficient to explain rotation resistance. O'Neal et al. (2002a) postulated that, as corn matures and becomes a less attractive and acceptable food, *D. v. virgifera* dispersal increases. In areas dominated by corn-soybean rotation, corn is typically planted early and with a high degree of synchrony. In this situation, emigrating beetles would predominantly encounter fields of equally unattractive corn or, alternatively, a soybean field.

Oviposition in the absence of a larval host plant has been recorded for *D. v. virgifera* (Kirk et al. 1969, Gustin 1979, Branson and Krysan 1981, Siegfried and Mullin 1990), and oviposition is not considered to be dependent upon cues from the larval host plant. Oviposition can occur without the presence of odor or physical cues from corn, even when females are feeding on or around plants that reduce adult longevity (Siegfried and Mullin 1990). These examples of oviposition in the absence of corn (Kirk et al. 1969, Gustin 1979, Branson and Krysan 1981, Siegfried and Mullin 1990) predate the occurrence of rotation resistance and were reported outside the current range of rotation-resistant *D. v. virgifera*. In the absence of clear evidence for an attraction to soybean by wild-type *D. v. virgifera*, adult emigration from cornfields remains a viable explanation for oviposition in soybean.

Emigration by adult *D. v. virgifera* from cornfields is common. Adults, especially females with mature eggs, have been observed to move from fields where they emerge and enter rotated cornfields not having an existing population (Godfrey and Turpin 1983). Like most *Diabrotica* species, *D. v. virgifera* prefers to feed on floral parts (Krysan 1986), and floral volatiles have been shown to be highly attractive (Metcalf 1986). Later planted (i.e., phenologically younger) corn that flowers later than surrounding

cornfields can function as a trap crop drawing in large numbers of adult corn rootworms (Hill and Mayo 1974, Naranjo and Sawyer 1994). Greater numbers of adult *D. v. virgifera*, especially females are found in late-planted cornfields than surrounding early planted cornfields (Darnell et al. 2000), and individuals have a greater tendency to fly toward corn of a younger phenological stage (Naranjo 1994). Volatile components of corn identified as attractants (Metcalf 1986, Hammack 1997) are produced by plants during pollination when silks and tassels are present. These observations suggest *D. v. virgifera* commonly move in and between cornfields seeking plants of the most favorable phenological stage for adult feeding. In a landscape of concentrated corn-soybean rotation where corn is planted with a high degree of synchrony, conditions are conducive for the deposition of *D. v. virgifera* in soybean.

The initial objective of the present research was to determine whether the response of rotation-resistant *D. v. virgifera* to corn phenology was greater than that of wild-type beetles. We assayed the propensity of adults from the two populations to leave corn during and after anthesis by using a two-chamber emigration arena. We further investigated whether adult attraction to soybean was influenced by corn and soybean volatiles. Using an olfactometer designed after Sammons et al. (1997), we tested whether adult attraction to soybean volatiles differed for these populations. We then documented how attraction to soybean volatiles was influenced by presence of corn at various growth stages.

## Materials and Methods

**Insects.** Beetles used for experiment 1 were obtained from a laboratory colony maintained at the Northern Grain Insects Laboratory, Brookings, SD. Emerging teneral adults collected over 48 h were sent overnight to Michigan in containers with food (butternut squash and a fructose-glycerine diet) and water. Beetles were delivered on 16 April 2001. Assayed beetles were  $\approx 14$  d old based on adult emergence occurring the day of shipment. These beetles were only used in experiment 1 to validate the design of the emigration assay apparatus. Conclusions based on experiments with laboratory-reared beetles were limited to general inferences about the experimental apparatus and design.

Experiments 2-5 used beetles collected in east central Illinois and Nebraska. Beetles from Nebraska, where injury to rotated corn has not been reported, were defined as wild type. Beetles from Illinois, where injury to rotated corn is common, were considered rotation-resistant. To better relate our study to that of Sammons et al. (1997), we used *D. v. virgifera* collected from cornfields in Nebraska (Saunders County, 16 July 2001 and 12 August 2002) 4 km from the collection site of Sammons et al. (1997). Nebraska beetles were shipped to Michigan in containers with corn ears, silks, water, and arrived within 48 h of collection. Adults from Illinois were collected from a

soybean field to ensure the sample population was composed of females likely to oviposit therein. We selected a collection site in Champaign County well known for injury to rotated corn (Spencer et al. 1999, O'Neal et al. 2001, Levine et al. 2002) to optimize the probability of collecting rotation-resistant beetles. Beetles were transported by car in cages with corn ears, silks, and water on 14 July 2001, and 12 and 26 August 2002.

All beetles from both states were kept in wire screen (30 by 30 by 30-cm) cages in an insect-rearing facility (photoperiod of 16:8 [L:D] h, 24°C) on the campus of Michigan State University. Water was provided ad libitum from dental wicks placed in water-filled flasks. Beetles were provided ears and tassels of both vegetative and reproductive-stage corn and soybean foliage to ensure that exposure to each stage and plant in the emigration assay and olfactometer was not a novel experience. In both the emigration and olfactometer experiments, individual beetles were only tested once. A subsample of beetles was preserved as voucher specimens in the A. J. Cook Arthropod Research Collection at Michigan State University.

Plants. Corn (Pioneer 3573) was grown at the Michigan State University Entomology Research Farm (East Lansing, MI) and maintained using conventional agronomic practices. In 2001 and 2002, we planted soybean (Asgrow Ag2201) and corn the first week of May, and corn every 7–10 d after that until four plantings were established to ensure a supply of vegetative and reproductive-stage corn for the experimental comparisons. All soybean used in the following experiments were from plants in the early reproductive stage (R4 to R5 stage); all plants had flowers and pods varying from 2 to 5 cm in length. Soybean foliage was presented in the olfactometer as four trifoliates cut  $\approx$ 30 cm from the leaf base and placed in a 50-ml Erlenmeyer flask filled with water.

Tests of adult *D. v. virgifera* propensity to depart from corn leaves in the emigration assay used corn in early vegetative stage (V6–V8 stage, Ritchie et al. 1986 scale, designated “young leaves”) or late vegetative stage (V14–VT stage, Ritchie et al. 1986, designated “old leaves”). Emigration and olfactometer assays used corn from the four planting dates for plants at anthesis (VT–R1 stage, Ritchie et al. 1986) and after anthesis (R3 to R4 stages, Ritchie et al. 1986). These two stages of corn are designated young and old, respectively. Tassels cut from early reproductive stage corn (VT, young tassels) were used only while producing pollen. Conversely, tassels from late reproductive stage corn (R2, old tassels) were used only when no longer producing pollen.

**Emigration Assay.** An emigration assay arena was constructed with wire screen (7 by 7 strands per square centimeter aluminum mesh) and a cardboard cylinder bottom (Fig. 1), divided into an inner and an outer section. To ensure that beetle emigration included flight, the inner wall of the outer section included a 10-cm Fluon-coated band that beetles could not climb. We measured adult *D. v. virgifera* emigration from the inner section when in the presence of

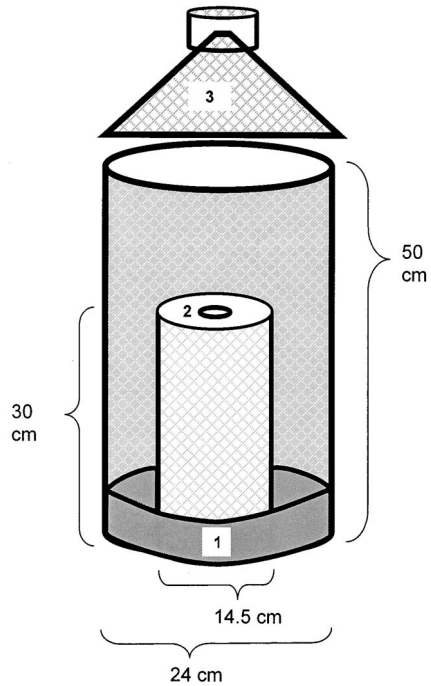


Fig. 1. Diagram of the emigration assay arena. 1, outer chamber with Fluon-coated aluminum foil covering the lowest 10 cm of the inner wall. 2, inner section with a 4.5-cm hole in its clear plastic top. 3, cone-shaped removable top of outer section.

corn of differing phenologies. The cone-shaped lid of the outer section opened into a clear plastic container (11 cm in height by 8 cm in diameter) to isolate beetles that emigrated from the inner section. Within the inner section, beetles were offered corn leaves or tassels placed within a water-filled, 50-ml Erlenmeyer flask, sealed at its mouth with Parafilm to prevent beetles from entering the flask. Both tassels and leaves were cut so that 5 cm was sealed within the flask and 45 cm extended from the flask. Beetles taken from stock cages were briefly chilled for easier handling and then separated by sex based on the shape of the last abdominal sternite (White 1977). Ten female beetles were placed within the inner section covered with a clear plastic lid. After 60 min, the lid was removed and the outer section sealed. We counted the number of adults within the inner section after 30 min, 3 h, and 24 h. Assays were set up at noon, so that beetles were undisturbed during times of peak flight activity in the early evening and morning (Witkowski et al. 1975). All assays were conducted at room temperature ( $23 \pm 3^\circ\text{C}$ ) and a photoperiod of 16:8 (L:D) h. The location of each emigration assay was randomly assigned within a grid on the bench within the laboratory.

**Experiment 1: Emigration Assay Validation.** During spring 2001, we tested our emigration assay with laboratory-reared *D. v. virgifera*; subsequent studies used only field collected beetles (experiments 2–5). We tested whether propensity of adult females to leave

the inner section was related to the corn tissue presented. We compared the retention of *D. v. virgifera* on a surrogate leaf to that of corn tissue (leaf or tassels). This surrogate was constructed from a 6 by 40-cm leaf-shaped section of smooth green cardboard similar in surface area ( $105 \text{ cm}^2$ ) to the leaf treatments. In each assay, beetles were offered one of five treatments (surrogate leaf, young corn leaf, old corn leaf, young tassel, and old tassel); the experimental design was a randomized complete block with five replicates. After 24 h, we counted the number of beetles remaining in the inner section of each assay. Treatment effects were analyzed using analysis of variance (ANOVA) followed by least significant difference (LSD) for mean separations (SAS Institute 2000). The amount of leaf area eaten during experiment I was also measured (O'Neal et al. 2002b) for the two leaf treatments. The difference in amount of leaf area consumed between the two leaf treatments was compared using Student's *t*-test with Satterthwaite correction (SAS Institute 2000).

**Experiment 2: Effect of Beetle Origin on Emigration.** Using beetles collected in the field during the summer of 2001, we looked for differences in behavior of rotation-resistant versus wild-type *D. v. virgifera* in the emigration arena. Based on the response of adults to corn phenology (O'Neal et al. 2002a), we postulated that Illinois beetles would have a greater propensity to leave corn of an advanced phenology than beetles from Nebraska. Old and young tassels were used to represent plants of varying phenologies. Both were tested simultaneously for 24 h; there were seven replicates of each combination of treatment and population of origin. We did not compare young or old corn leaves, because preliminary tests found no difference in the number of Illinois or Nebraska beetles in inner chambers containing these treatments (O'Neal 2003).

We analyzed data in two separate analyses. The first accounted for the effect of beetle origin on emigration using ANOVA for a three-way factorial design, with population of origin, time (beetles in inner section after 30 min, 3 h, and 24 h), and plant tissue as treatment factors. The second analysis determined whether both populations preferred either old or young tassels. A mean observed ratio of beetles in the inner to the outer section was calculated for each population of origin and plant tissue combination and compared via chi-square goodness-of-fit test (Sokal and Rohlf 1995) to a 1:1 ratio, indicating no preference.

**Olfactometer.** We modified the olfactometer (Fig. 2) of Sammons et al. (1997) composed of a series of satellite chambers connected to a central chamber by side-arms fashioned from flexible clear tubing (1.7 cm interior diameter, 11.5 cm in length; Tygon, Fisher Scientific, Pittsburgh, PA). Modifications included a smaller central chamber (8 cm in height, 18.2 cm in diameter), four rather than eight satellite chambers (19 cm in height, 20.0 cm in diameter; TriState Plastics, Dixon, KY), and a sponge (9 by 5.5 cm) saturated with water in each satellite chamber to control for humid-

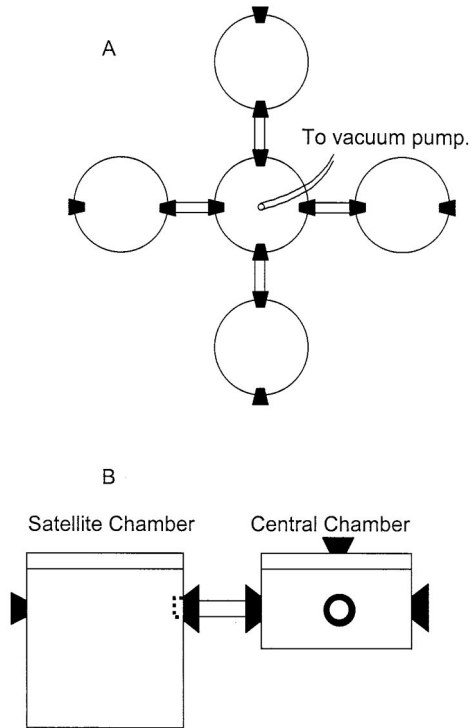


Fig. 2. Olfactometer design from (A) top view and (B) side view.

ity. To ensure that beetles responded only to volatiles emanating from satellite chambers, the central chamber was wrapped with white cardboard blinds to block any visual cues. Air flowed at a velocity of  $0.5 \pm 0.06$  liters/min through satellite chambers to the central chamber and then to a vacuum pump (Gast Mfg. Corp., Benton Harbor, MI) attached to the top of the central chamber. The intake hole (3.2 mm in diameter) of each satellite chamber was gauze-covered and opposite the opening to the central chamber (Fig. 2). All olfactometer tests were conducted at 21–24°C with a photoperiod of 16:8 (L:D) h, by using female *D. v. virgifera*. We released 20–25 female beetles in the central chamber at 1400 hours and recorded their position after 22 h. Before an olfactometer was used, all connecting tubes, inner and outer chambers were washed with soap, rinsed, and dried. The sponges were triple-rinsed and soaked overnight in warm water and filled for use with distilled water.

We conducted preliminary tests to determine the likelihood of beetle movement from satellite chambers to the central chamber. Fifty beetles were placed in a satellite chamber with only a sponge; only one beetle moved to the empty central chamber in 24 h. We conclude that beetle movement was unidirectional, from the central toward the satellite chambers. Beetles were counted in a satellite chamber if they had moved into the tube connected to the outer chamber. During all olfactometer experiments <3% of beetles

tested were found in the tubes connecting the central and satellite chambers.

**Experiment 3: Attraction of Illinois and Nebraska Beetles to Soybean.** To determine whether soybean volatiles were attractive to adult *D. v. virgifera*, we placed cuttings of soybean foliage and a wet sponge in one randomly selected satellite chamber of the olfactometer. The other three received only a wet sponge. Both populations were tested in two separate olfactometers on three consecutive days for a total of six replicates per population. We summed the number of beetles in each type of satellite chamber and compared the observed distribution to an expected random distribution (0.25:0.75, soybean and sponge; sponge only ratio) by using chi-square goodness-of-fit test. In a separate analysis with the same data, we determined whether the responses of Nebraska and Illinois beetles differed. We summed the total in each chamber type across the three days and compared the distribution of beetles from Illinois to those from Nebraska by using a chi-square goodness-of-fit test.

**Experiment 4: Attraction of Illinois Beetles to Soybean in the Presence of Old Corn Volatiles.** Two satellite chambers contained a tassel and ear of old corn, whereas the remaining two chambers contained soybean foliage. Four replications were run on three separate days, and the location of the plant materials was randomized daily. We summed the number of beetles in each of the satellite chambers and compared the observed distribution to an expected random distribution (1:1, old corn:soybean ratio) by using chi-square goodness-of-fit test.

**Experiment 5: Effect of Varying Corn Phenology on Attraction of Illinois Beetles to Soybean.** Each of the satellite chambers contained a water-filled sponge and three chambers received one of the following; young corn (tassels and an ear from a plant in anthesis), old corn (tassels and an ear from a postanthesis plant), and soybean. Two configurations of the olfactometer were conducted simultaneously. In the first configuration, all four materials were placed in separate satellite chambers. In the second configuration, the chamber containing the young corn was replaced with a chamber containing old corn. Each configuration was tested simultaneously in two replicates on three consecutive days. The location of the plant materials was randomized daily.

We used a chi-square goodness-of-fit test to determine whether the distribution of beetles in the first configuration (both young and old corn present) differed from that of the second configuration (only old corn present). The distribution of beetles in the first configuration was used to calculate an expected distribution for the second configuration. The expected number of beetles in the old corn of the second configuration was calculated by adding the number of beetles in chambers with old and young corn from the first configuration, dividing by the total tested in the first configuration, and then multiplying by the total tested in the second configuration.

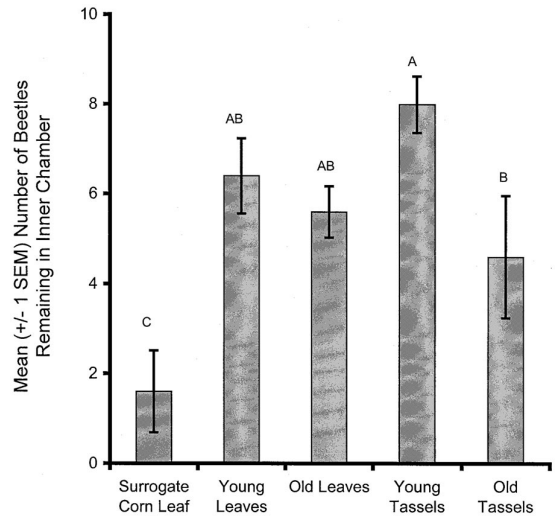


Fig. 3. Emigration response after 24 h by *D. v. virgifera* from a laboratory colony to young corn leaves (mid-vegetative stage), old corn leaves (reproductive stage), young tassels (shedding pollen), old tassels (no pollen), and a surrogate leaf. Bars with same letter are not significantly different according (LSD,  $P \leq 0.05$ ).

## Results

**Experiment 1: Emigration Assay Validation.** Types of corn tissue or surrogate leaf significantly ( $F = 7.6$ ,  $df = 4, 24$ ;  $P = 0.001$ ) affected emigration of adult female *D. v. virgifera*. The fewest females were found in inner sections with the surrogate leaf (Fig. 3); no beetles remained inside the inner section in two of the five replicates. Young tassels shedding pollen retained more females than old tassels. Although there was no difference in emigration from young and old corn leaves ( $P = 0.05$ ; Fig. 3), a significantly greater leaf area was consumed ( $t = 2.78$ ,  $df = 4$ ,  $P = 0.05$ ) by females on the young leaf ( $7.94 \pm 2.62$ , mean  $\text{cm}^2 \pm \text{SEM}$ ) than the old leaf treatment ( $1.29 \pm 0.54$ ).

**Experiment 2: Effect of Beetle Origin on Emigration.** As described above, significantly greater emigration occurred when beetles were presented with old than young tassels (Fig. 4), but the origin of the beetles did not affect emigration ( $P = 0.74$ ; Table 1) at any of the observation times ( $P = 0.71$ ; Table 1). There was only one significant interaction (time by plant,  $P < 0.01$ ; Table 1), but it was not associated with beetle population of origin. We attribute this interaction to the lack of significant differences in beetle emigration from the inner sections until the final time period (Fig. 4), which was the case for both populations.

Beetle distribution between the inner and outer sections differed significantly from an expected 1:1 ratio in assays where old tassels were presented (Table 2). However, the distribution did not differ from a 1:1 ratio when young tassels were presented to beetles. Beetles from Illinois and Nebraska yielded the same pattern.

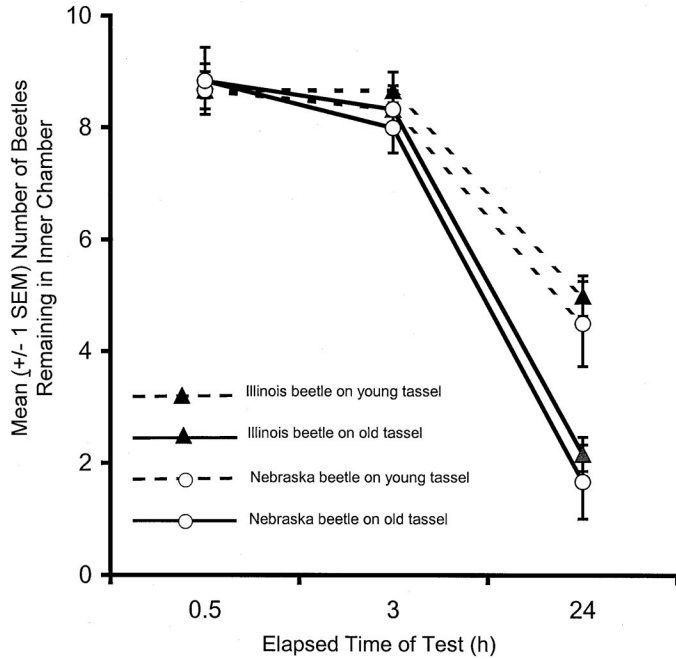


Fig. 4. Emigration response after 30 min, 3 h, and 24 h by *D. v. virgifera* from Nebraska and Illinois to young tassels (shedding pollen) and old tassels (no pollen).

**Experiment 3: Attraction of Illinois and Nebraska Beetles to Soybean.** On a per chamber basis, more beetles from both populations moved to satellite chambers with soybean than satellite chambers with only a sponge (Table 3). There was no difference in response of beetles to soybean due to population of origin ( $P = 0.69$ ; Table 3).

**Experiment 4: Attraction of Illinois Beetles to Soybean in the Presence of Old Corn Volatiles.** Beetle distribution in olfactometers with two satellite chambers containing soybean foliage and two chambers containing old corn ears and tassels was not evenly distributed ( $\chi^2 = 19.95$ ,  $df = 1$ ,  $P < 0.0001$ ); nearly twice as many female beetles moved to chambers containing old corn than soybean (total in old corn = 146, total in soybean = 79). Thus, even old corn was more attractive than soybean.

**Experiment 5: Effect of Varying Corn Phenology on Attraction of Illinois Beetles to Soybean.** With young and old corn in the olfactometer (first configuration), more beetles found the young corn (Table 4). When

the young corn was replaced with old corn (second configuration), more beetles were recovered in chambers containing corn than either of the noncorn chambers. However, the distribution of beetles across the four chambers differed between the first configuration containing both young and old corn and the second configuration containing only old corn ( $\chi^2 = 28.6$ ,  $df = 1$ ,  $P < 0.01$ ; Table 4). Between the two configurations, the percentage of beetles found in the chambers without corn (soybean and sponge alone) doubled (13% in the first configuration versus 27% in the second configuration).

Discussion

**Alternative Explanatory Models for Rotation Resistance.** Various explanations can be advanced to account for the recent failure of corn-soybean rotation to manage *D. v. virgifera*. A change in *D. v. virgifera* behavior ultimately leading to increased oviposition in

Table 1. Comparison of Illinois and Nebraska *D. v. virgifera* emigration from corn tissue of varying phenology at 30 min, 3 h, and 24 h after placement on plant

Source	df	F	P
Beetle origin	1	1.05	0.32
Time	2	141.38	<0.01
Plant	1	17.66	<0.01
Time*Origin	2	0.35	0.71
Origin*Plant	1	0.12	0.74
Time*Plant	2	7.37	<0.01
Time*Origin*Plant	2	0.27	0.76

Table 2. Distribution of female *D. v. virgifera* after 24 h in emigration arenas presenting corn tassels of varying phenologies

State of beetle origin	n	Tassel age <sup>a</sup>	Observed ratio (inner:outer chamber)	$\chi^2$	P
Illinois	70	Old	1.8:8.2	3.94	0.04
		Young	3.5:6.5		
Nebraska	70	Old	1.7:8.3	4.36	0.04
		Young	5:5		

Data derived from experiment 2, Fig. 4.

<sup>a</sup> Young tassels were taken from plants producing pollen; old tassels were taken from plants 2 wk passed the pollination period and no longer shedding pollen.

**Table 3.** Distribution of female *D. v. virgifera* after 22 h in a four-chamber olfactometer

State	Satellite chamber content <sup>a</sup>	No. of beetles		Comparison within a population	
		Observed <sup>b</sup>	Expected <sup>c</sup>	$\chi^2$	P
Illinois	Soybean	42	24.5	16.7	<0.01
	Sponge	56	73.5		
Nebraska	Soybean	54	27.25	37.5	<0.01
	Sponge	55	81.75		
Comparison between populations <sup>d</sup>				0.15	0.69

<sup>a</sup> Each olfactometer was arranged with one satellite chamber containing soybean foliage and sponge and three containing only a sponge.

<sup>b</sup> Total beetles in each treatment (soybean or sponge) is summed from two replicates on three consecutive days in which 20 to 25 beetles were released per olfactometer.

<sup>c</sup> Expected number of beetles is calculated for comparing distributions of beetles within an olfactometer assuming no attraction to soybean and an equal distribution across the four chambers resulting in a 0.25:0.75 ratio.

<sup>d</sup> Comparison between populations was performed by comparing the distribution of Illinois (observed) to Nebraska (expected) beetles.

soybean has been considered the most likely mechanism by which beetles thrive in a rotated corn-soybean landscape (Levine et al. 2002). Hence, the following discussion focuses only on explanatory models invoking behavioral change. Nevertheless, other causal mechanisms, such as a delay in the emergence of adult *D. v. virgifera* (O'Neal 2003), should be considered along with those below.

A genetically based behavioral change model has been offered to explain increased *D. v. virgifera* oviposition in soybean (Sammons et al. 1997; Onstad et al. 1999, 2001; Levine et al. 2002). Our interpretation of this conceptual model is that a change in the behavior of *D. v. virgifera* in east central Illinois would lead to an increase of injury to rotated corn and that this modified behavior has a genetic basis. The report by Sammons et al. (1997) that Illinois beetles were more attracted to soybean than were Nebraska (wild-type) beetles provided initial evidence for the genetically

based behavioral change model. Unable to corroborate the Sammons et al. (1997) result, Spencer et al. (1999) proposed that decreased fidelity for corn could be the altered behavior arising from the selection pressure of a corn-soybean rotated landscape. This new behavior was thought advantageous to rotation-resistant *D. v. virgifera* populations, and its occurrence was explained by a single-locus genetic model (Onstad et al. 2001). A logical prediction of the genetically based behavioral change model is that the genotype would spread geographically like any adaptive trait (e.g., insecticide resistance gene) carried by insects with substantial capacity to disperse by flying. Onstad et al. (1999) suggested the geographic spread in the occurrence of large numbers of *D. v. virgifera* in soybean has been consistent with the spread expected for a rotation resistance gene arising in east central Illinois. An additional prediction of this model would be that rotation resistance is irreversible or at best slowly reversible once the variant genotype becomes fixed in a given geographical location with a susceptible landscape structure.

Based largely on outcomes of the experiments presented here, we propose another possible explanation for the phenomenon of *D. v. virgifera* oviposition in soybean. The phenological asynchrony behavioral plasticity model acknowledges increased oviposition in soybean in areas of strict corn-soybean rotation. However, we suggest the altered behavior is caused by a decrease in attractiveness of corn relative to soybean that, in turn, is due to changing agricultural practices (early planting, variety selection) that result in more phenologically mature corn at an earlier time in the growing season. Thus, when adult *D. v. virgifera* are most abundant at their usual developmental time, corn in a more advanced phenological state attracts and retains fewer beetles. Like the genetically based behavioral change model, the phenological asynchrony behavioral plasticity model predicts increased beetle visitation of and oviposition in soybean, leading to corn damage the following season. But, the phenological asynchrony behavioral plasticity model differs from the genetically based behavioral change model

**Table 4.** Distribution of female *D. v. virgifera* after 22 h in a four-chamber olfactometer with and without young corn

Configuration	Satellite chamber content	No. of beetles		No. of beetles expected <sup>b</sup>
		Mean ( $\pm$ SEM)	Observed <sup>a</sup>	
First configuration	Young corn	10.0 $\pm$ 2.1	70	83
	Old corn	2.9 $\pm$ 0.6	20	
	Soybean	1.8 $\pm$ 0.8	11	
	Sponge only	0.5 $\pm$ 0.3	3	
Second configuration	Old corn	6.0 $\pm$ 1.3	70	10
	Soybean	3.0 $\pm$ 1.0	18	2
	Sponge only	1.3 $\pm$ 0.5	8	

<sup>a</sup> Total beetles in each configuration is summed from two olfactometers on three consecutive days in which 20 to 25 beetles were released.

<sup>b</sup> Expected number of beetles is calculated based on the distribution observed in the first configuration. The number of beetles observed in the young and old corn chambers combined to calculate an expected old corn observation for the second configuration. There was a significant difference between the observed and expected distribution for the second configuration ( $\chi^2 = 28.6$ , df = 1,  $P < 0.01$ ).

by not requiring a genetic shift in *D. v. virgifera* genotype. Rather, behavior inherent within the bounds of behavioral plasticity of the original genotype is sufficient to increase oviposition in soybean. The phenological asynchrony behavioral plasticity model predicts 1) no difference in the behaviors of Illinois and Nebraska *D. v. virgifera* encountering identical corn or soybean stimuli; 2) the timing of and geographical pattern for spread of rotation failure would be highly correlated with that for landscape structure permitting its expression; and 3) rotation failure would be reversible upon reversion to the landscape structure and phenological synchrony that preceded *D. v. virgifera* injury to rotated corn.

**Fit of Current Data to Respective Models.** This study was directed both at whether differences could be found between plant-induced behaviors of Illinois (rotation-resistant) versus Nebraska (wild-type) *D. v. virgifera*, and whether beetles responded differently to corn varying in phenological state. Although the emigration assay detected large differences in behavioral responses to surrogate versus authentic plant material (Fig. 3) and clear differences between young and old corn tassels (Fig. 4; Table 1), we found no effects of beetle origin (Table 1).

Our side-arm olfactometer tests revealed Illinois and Nebraska beetles were equally attracted to soybean (Table 3). This outcome contradicts the results of Sammons et al. (1997) who reported that Illinois beetles were 2.7-fold more attracted to soybean than were beetles from Nebraska. This difference in outcomes is particularly striking because both studies used the same apparatus, and a similar population to represent wild-type *D. v. virgifera*. However, we did modify the design and protocol of Sammons et al. (1997) olfactometer by reducing the number of satellite chambers, only using plant material, and testing only female beetles. We suggest that these modifications should have aided in measuring an attraction for soybean by rotation-resistant *D. v. virgifera*. Our only significant departure in methodology was inclusion a water-saturated sponge in the satellite chambers to control for relative humidity. The current findings, considered together with those of Spencer et al. (1999) and O'Neal et al. (2002a), leads to the tentative conclusion that there currently are no data directly supporting a significant genetic-based shift of behavior. The indirect evidence of Onstad et al. (1999, 2001) for the genetically based behavioral change model is not discounted. But, evaluation of the strength of this indirect evidence based on pattern of geographic spread of rotation failure awaits partitioning of that effect between two possible causes: spread of agricultural practices (reduced diversity in the landscape and changes in corn phenology) that allow expression of preexisting behavior versus spread of a resistant genotype.

Recognizing the potential high impact of phenological asynchrony between beetles and corn, O'Neal et al. (2002a) built on Spencer et al.'s (1999) proposal that a rotation-resistant *D. v. virgifera* population might differ from the wild type in heightened propensity to

leave old corn, i.e., reduced corn fidelity. However, the current data are incongruent with this hypothesis; emigration of Illinois beetles was not greater than that of Nebraska beetles in our laboratory assay (Fig. 4). The response of both beetle populations was consistent with the mechanism proposed by O'Neal et al. (2002a) for oviposition of *D. v. virgifera* within soybean fields. That is, adults readily emigrate from corn at an advanced phenological state to whatever fields are nearby. In fact, slightly more than half of the beetles placed in the inner chamber of emigration assay with corn of an optimal phenological state left the optimal host and moved to the outer chamber within 24 h. For old corn, the ratio of beetles leaving versus staying in the inner chamber was >4:1. Thus, old tassels are indeed less arrestive than young tassels, lending credence to the idea that corn phenology can influence beetle movement.

Unable to identify a rotation-resistant *D. v. virgifera* as evidenced by greater emigration from old corn (Fig. 4; Table 2), we quantified the attraction of Illinois beetles to young and old corn relative to soybean (Table 4). Expressed as fold increase over response to a wet sponge, attractiveness of young corn, old corn, and soybean was 23, 6.6, and 3.7, respectively. Equivalent values for the test with only old corn and soybean presented with a wet sponge (Table 4) were 4.4 and 2.2, respectively. Thus, soybean seems to be approximately one-half as attractive as old corn, but less than one-sixth as attractive as young corn in the side-arm olfactometer. Moreover, when young corn was removed as one of the choices, leaving only old corn as host material (experiment 5), the number of beetles collected in chambers with a nonhost (soybean or sponge) rose significantly. We conclude that volatiles from old corn are indeed less attractive to adult *D. v. virgifera* than volatiles from young corn and that substituting old for young corn could increase visitation to nearby soybean plants.

Collectively, our data are not supportive of the genetically based behavioral change model for rotation failure. No direct evidence was obtained for any difference in behavior of Illinois versus Nebraska *D. v. virgifera*. Current data are consistent with the phenological asynchrony behavioral plasticity model, which is based on inherit behavioral plasticity of beetles from both populations. Differentiating between these explanatory models awaits either 1) convincing evidence for meaningful genetic change between Illinois versus wild-type beetles, or 2) testing of new predictions from the genetically based behavioral change and phenological asynchrony behavioral plasticity models that do not overlap. A prediction consistent with the phenological asynchrony behavioral plasticity model and inconsistent with the genetically based behavioral change model is reversal of rotation resistance if synchrony of adult *D. v. virgifera* peak activity with optimal corn phenology for adults' is restored.

The initial geographic spread of *D. v. virgifera* displaying behaviors associated with injury to rotated corn (Onstad et al. 1999) can be used as evidence

supporting the genetically based behavioral change model. Recently, the role of reduced landscape diversity has been shown to play a significant role in determining the range of *D. v. virgifera* displaying behaviors associated with rotation resistance (Onstad et al. 2003). The practice of a corn-soybean rotation is very high in east central Illinois, a region whose farm landscape is >90% corn and soybean (USDA Census of Agriculture data summarized in Onstad et al. 2003). This lack of landscape diversity is unique to east central Illinois and can help explain why injury to rotated corn originated in this part of the state. However, landscape diversity alone does not explain how *D. v. virgifera* has come to use soybean as an oviposition site. A corollary to our last prediction is that the agroecosystem of a region where injury to rotated corn is common, such as east central Illinois, would also have a high degree of asynchrony between corn and *D. v. virgifera* phenology. As suggested by the phenological asynchrony behavioral plasticity model, a combination of reduced landscape diversity and asynchrony between corn and *D. v. virgifera* phenology is required to produce significant *D. v. virgifera* oviposition in soybean. To what degree the synchrony between corn and *D. v. virgifera* phenology in east central Illinois differs from that of the rest of the Corn Belt is not well understood.

Until such definitive data are obtained, we would argue that the phenological asynchrony behavioral plasticity model realizes an advantage over the genetically based behavioral change model due to simplicity. Both models invoke changes in landscape structure over time. However, the phenological asynchrony behavioral plasticity model does not require a genetic transformation in the beetle population; the mechanism of phenological asynchrony evoking already inherent behaviors seems sufficient to explain the phenomenon. For these reasons, we urge that both of these models be given due consideration, along with possible unforeseen alternatives, because answers are sought to the phenomenon of *D. v. virgifera* injury to rotated corn.

### Acknowledgments

We gratefully acknowledge Lance Meinke and Joseph Spencer for providing western corn rootworms from Nebraska and Illinois, respectively. We thank Larry Bledsoe and Richard Edwards for use of a side-arm olfactometer. We thank Mike Brewer and Piera Giroux, and two anonymous reviewers for providing helpful comments on earlier versions of this manuscript. We thank Rufus Isaacs, Donald Sebolt, Mike Jewett, Alison Gould, Megan Burns, Kevin Newhouse, and Andre Ball for technical assistance. Funding was provided by USDA-NRI Entomology-Nematology grant 01-35302-10965 and the C.S. Mott chair for sustainable agriculture at Michigan State University.

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*Received for publication 11 April 2003; accepted 10 October 2003.*

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