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dissertation entitled

IS CORN PHENOLOGY RESPONSIBLE FOR THE FAILURE OF CROP ROTATION TO MANAGE WESTERN CORN ROOTWORMS (*DIABROTICA VIRGIFERA VIRGIFERA* LECONTE)?

presented by

Matthew Elliott O'Neal

has been accepted towards fulfillment of the requirements for the

Ph.D. degree in Entomology

Major Professor's Signature

4/14/03

Date

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By

Matthew Elliott O'Neal

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

2003

ABSTRACT

IS CORN PHENOLOGY RESPONSIBLE FOR THE FAILURE OF CROP ROTATION TO MANAGE WESTERN CORN ROOTWORMS (DIABROTICA VIRGIFERA VIRGIFERA LECONTE)?

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Western corn rootworm (*Diabriotica virgifera virgifera* LeConte) have expanded their oviposition range to include soybean fields where a corn-soybean rotation is routinely practiced to disrupt this insects' life cycle. Rotation-resistance is apparently spreading, although surveys of Michigan soybeans from 1999-2001 did not reveal adult *D. v. virgifera* populations in soybeans that were consistently above action thresholds. To confirm if rotation-resistant *D. v. virgifera* are present in Michigan, I investigated behavioral features that contribute to an adaptation to crop rotation. By measuring leaf feeding, an influence of corn phenology on the consumption of soybean leaf by *D. v. virgifera* was observed, but did not differ between putative rotation-resistant and wild type populations. Phenology-mediated dispersal from corn could not separate putative variant from wild type *D. v. virgifera.* Although no difference was observed between rotation-resistant and wild type populations, a consistent trend for increasing corn maturity to favor adult acceptance of soybean has emerged. I suggest synchronous and earlier corn planting combines with landscape structure to favor oviposition of *D. v. virgifera* in soybean. Under these conditions, rotation-resistance is an emergent property that all populations may display. While genetic change resulting in a rotation-resistant population may ultimately occur, it is not a pre-requisite. This insight has important implications for both the evolution of pest life histories and rootworm management.

A child's world is fresh and new and beautiful, full of wonder and excitement. It is our misfortune that for most of us that clear-eyed vision, that true instinct for what is beautiful and awe-inspiring, is dimmed and even lost before we reach adulthood.

Rachel Carson

ACKNOWLEDGEMENTS

I would like to say that I was clever enough to design all the experiments and sufficiently industrious to perform them on my own. But I should be humble enough to admit that none of this was possible without the help of everyone in the Entomology Department.

The following work was conducted under much-appreciated, heavy guidance of co-advisors Drs. Douglas Landis and Christina DiFonzo. Their complementary roles provided me with a full, rich doctoral program that went far beyond professional development. I am very thankful for the involvement of my committee members, Drs. Dick Harwood, Rufus Isaacs and Stuart Gage. Each gave generously of their time and experience and provided me with insights for the research presented here and the development of my career as a scientist. Though not officially a member of my committee, Dr. Jim Miller is an irreplaceable mentor whose openness and generosity of time is inspiring. It is not possible to thank him fully, and maybe the only appropriate way is to pass on the kindness shown to me.

For every experiment, I am in debt to many people for their technical assistance. Mike Haas, Mike Jewett and Chris Sebolt provided year round, irreplaceable assistance. Each gave their help with much patience and unwavering support. David Meeks, as an undergraduate at Kalamazoo College (Kalamazoo, Michigan), was an industrious summer research assistant. His collaboration during the summer of 2000 was invaluable for collecting *D. v. virgifera* emergence data and led to the successful completion of his Senior Thesis. During the course of my doctoral program I was very fortunate to work with many undergraduate student employees. All of the following provided much needed mental and physical support: Andre Ball, Meghan Burns, Chris Cerveny, Sandra Clay, Alison Gould, Matt Leonard, Alison Lewinsky, Cathy Mccamant, Ben Nessia, Kevin Newhouse and Tim Schutz.

I am indebted to many who volunteered their time, fields and logistic help that made the 'on-farm' and field work possible. Though it is not possible to name all of the farmers and extension agents that helped in this work, the following stood out for their consistent source of assistance: Pat Feldpausch, the Wykoff family of Indiana, John Schumacher and Adoree Miron of Pioneer Seed Company of Constantine; Michigan extension agents Bruce McKellar and Ned Birkey.

I thank Drs. Joseph Spencer, Lance Meinke and the USDA-ARS Insectary at Brookings, South Dakota, for providing *D. v. virgifera*.

Financial support was gratefully received from Project GREEEN (Project numbers 9946, 0027), Corn Marketing Program of Michigan, and USDA NRI (project number 2001-3502-10965). This research has been supported by a Charles Stuart Mott Foundation Fellowship in Sustainable Agriculture.

Every scientist, no matter the subject, hopes that someone or something will benefit from their work. If a group of experiments that test a theory can improve the human condition, and something is to be reaped from the labors presented here, then I hope Elaine and at least our daughter receive that reward. Her patience and support deserve recognition.

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CHAPTER 1:

INTRODUCTION

Western corn rootworm (*Diabriotica virgifera virgifera* LeConte) have expanded their oviposition range to include soybean (*Glycine max* L.) fields and now cause significant damage where a corn (*Zea mays* L.) -soybean rotation is routinely practiced (Levine and Gray 1996, O'Neal et al. 2001). Injury to rotated corn (corn planted after soybean) has become common throughout much of the corn production region of the north central United States, resulting in the reversal of a decade-long reduction in soil insecticide use (Pike et al. 1991).

Onstad et al. (1999) described the initial occurrence and spread of injury to rotated corm by *D. v. virgifera*. Model results supported the hypothesis that a population of *D. v. virgifera* capable of circumventing a corn-soybean rotation originated in Ford County, Illinois (Levine and Oloumi-Sadeghi 1996). Furthermore, Onstad et al. (2001) used a model based on a single-gene locus for rotation-resistance to explain the occurrence of this population in east-central Illinois 16 years after the *D. v. virgifera* invaded Illinois. Efforts to separate rotation-resistant individuals from the wild type have not been successful (Spencer et al. 1999, Hibbard et al. 2002, O'Neal et al. 2002). Definitions of resistance, when used to describe the failure of an insecticide to control an insect pest, requires a genetic basis for the failure of the chemical to control a resistant population (Mota-Sanchez et al. 2002). The ability of *D. v. virgifera* to survive a corn-soybean rotation is referred to as an apparent resistance as, to date, no genotypic or phenotypic markers can separate the rotation-resistant from wild type populations.

By investigating factors germane to the behavior(s) which would influence *D. v. virgifera* to oviposit in soybean fields, both behavioral and physiological characteristics that could produced this variant's adaptation to crop rotation are characterized in this publication. Knowing the mechanism by which this apparent resistance to rotation developed is crucial for development of improved management strategies.

The current model for how *D. v. virgifera* became resistant to rotation emphasizes the widespread adoption of a corn-soybean rotation (Sammons et al. 1997, Spencer et al. 1999). Within a landscape that is predominately rotated between corn and soybean, a selective advantage is realized by *D. v. virgifera* that expand their egg-laying to include soybean fields. However, this model does not clearly state what features of a rotation-resistant *D. v. virgifera* would confer such a selective advantage within this landscape.

Initially, an attraction to soybeans by variant *D. v. virgifera* was predicted by Sammons et al. (1997). However, this was refuted, which led to the suggestion that movement out of cornfields is the behavioral basis for the subsequent failure of crop rotation (Spencer et al. 1999). Corn phenology was shown in prior studies to influence *D. v. virgifera* movement and oviposition in cornfields (Hill and Mayo 1974, Naranjo 1991), and may play a role in explaining how *D. v. virgifera* adaptation to a corn soybean rotation occurred. *D. v. virgifera* response to advancing corn phenology could be the primary factor resulting in dispersal out of cornfields. In landscapes dominated by corn-soybean rotations subsequent movement to, and oviposition in soybean fields results in increased survival and the potential for a positive feedback loop reinforcing selection for such dispersal traits. If this model is true, it has important implications for management of *D. v. virgifera* throughout the Corn Belt.

Rootworms and Crop Rotation; an IPM Success Story

Corn rootworms (*Diabriotica spp.*), are considered the primary pest of corn throughout the north central region of the United States (Krysan 1986, Levine and Oloumi-Sadeghi 1991). In the past, throughout most of the Corn Belt, corn was typically produced on the same field for many years in a row (continuous corn) as both a cash crop and a source of animal feed. As agriculture in the region shifted from animal production to grain production (Berlan 1991), corn and, later, soybean became the main cash crops. For the last 20 years, recommendations for corn rootworm management primarily involved a rotation of corn with a non-host crop. Throughout the 1980s, crop rotation was considered the most effective method for preventing corn rootworm injury (Levine and Oloumi-Sadeghi 1991, Gray and Luckmann 1994). Where crop rotation was adopted, a significant reduction in the use of soil insecticides was achieved (Pike et al. 1991). However, by the mid-1980s, damage by corn rootworm larvae to rotated corn (first-year corn) occurred throughout east central Illinois (Steffey et al. 1992). Most larval injury in the mid- to late 1980s was not economic and was initially attributed to various causes: the prolonged diapause of northern corn rootworm, *Diabrotica barberi* Smith and Lawrence (Chiang 1965, Landis et al. 1992, Steffey et al. 1992), high concentration of volunteer corn in soybean fields (Shaw et al. 1978), or to repellency of *D. v. virgifera* from pyrethroid-treated cornfields into adjacent soybean fields (Levine and Oloumi-Sadeghi 1996). During the early to mid-1990s, increase in the frequency and severity of *D. v. virgifera* larval damage to corn planted after soybeans raised concern that the extensive use of crop rotation had selected for a variety of *D. v. virgifera* that oviposited in soybeans (Levine and Gray 1996, Sammons et al. 1997, Spencer et al. 1999, O'Neal et al. 1999, O'Neal et al. 2001).

Hypothesis/Model for Rotation-Resistance of *Diabrotica virgifera*

In a corn-soybean rotation, larvae hatching from eggs that are oviposited into and overwinter in soybean, emerge in a cornfield the following spring. These first-year cornfields are typically not treated with soil insecticide and can experience extensive root injury and yield-reduction from *D. v. virgifera* larval feeding. Alternatively, in areas of intensive corn-soybean rotation, eggs laid in cornfields hatch the following year in a non-host field (i.e. soybean). Given that *D. v. virgifera* larvae do not feed on soybean, and eggs do not exhibit an extended diapause (Levine and Gray 1996, Levine and Oloumi-Sadeghi 1996), there appears to be a selective advantage for female *D. v. virgifera* to lay eggs in soybeans. Accordingly, O'Neal et al. (1999) found greater numbers of adult *D. v. virgifera* in soybean fields versus cornfields in east central Illinois. They also found a greater percentage of adult females in soybean fields than in cornfields during August (O'Neal et al. 1999), when *D. v. virgifera* likely lay most of their eggs (Hein and Tollefson 1985).

Both Sammons et al. (1997) and Spencer et al. (1999) observed suspected variant *D. v. virgifera* adults feeding on soybean leaves. Specifically, Sammons et al. (1997) reported that suspected variant beetles from Indiana fed more on soybean leaves in a no-choice assay than putative wild type *D. v. virgifera* from Iowa and Nebraska, both regions where rotation failures were not reported. They found that beetles from Nebraska did not feed on soybean leaves. Spencer et al. (1999) also reported field observations from Illinois of *D. v. virgifera* feeding on soybeans. Spencer et al. (1999) suggested that although a preference for soybeans is not necessary to explain rootworm oviposition in soybeans, it may be possible to differentiate wild type from variant *D. v. virgifera* by their propensity to feed on soybeans.

Oviposition by *Diabrotica virgifera*

Field and laboratory studies have consistently demonstrated that a damp, particulate substrate is sufficient to induce *D. v. virgifera* oviposition (Kirk et al. 1969, Gustin 1979, Branson and Krysan 1981, Siegfried and Mullin 1990). Kirk et al. (1969) concluded that *D. v. virgifera* had no inherent attraction to corn as an oviposition site. More recently, Siegfried and Mullin (1990) suggested that oviposition is not regulated by cues on or near the host plant, and that adult feeding and oviposition represent separate behaviors. Consequently, it appears that gravid *D. v. virgifera* females are likely to lay eggs where they happen to be feeding or resting.

Oviposition by *D. v. virgifera* in soybean fields is an example of seemingly deleterious or 'confused' oviposition (coined first by Larsson and Ekbom 1995) in a habitat containing a plant unsuitable for development of her offspring. This type of behavior is not unprecedented and examples have been observed in various species of Lepidoptera (Wiklund 1984, Janz and Nylin 1997), Cecidomiyids (Larsson and Ekbom 1995) and a Bruchid (Johnson and Siemens 1991). Oviposition preference is considered a key factor in the development of associations between phytophagous insects and their host plants (Thompson 1987, Jaenike 1990). Factors that influence oviposition are varied, but can often be traced to individual characteristics of the host plant (Miller and Strickler

1984). Host-quality (Larsson and Ekbom 1995, Janz and Nylin 1997) is critical in determining oviposition range of the searching female. Thus, understanding how host quality influences female dispersal and subsequent oviposition is critical in revealing how resistance to rotation occurred in *D. v. virgifera*.

The attractiveness of corn to *Diabrotica spp*. varies with plant age. Corn phenology was previously shown to influence northern corn rootworm dispersal (Naranjo and Sawyer 1988), and is thought to have a similar affect on *D. v. virgifera* movement (Naranjo 1991). *Diabrotica virgifera virgifera*, especially females with mature eggs, have been observed to move from fields where they emerge and enter rotated cornfields (Godfrey and Turpin 1983). Like most *Diabrotica spp., D. v. virgifera* prefer to feed on floral parts (Krysan 1986) and floral volatiles from these parts have been shown to be highly attractive (Metcalf 1986). It is also well known that later-planted (phenologically young) corn, which 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 1988). These observations suggest *D. v. virgifera* commonly move in and between cornfields in a crop landscape seeking plants of the most favorable phenological stage. However, the role corn phenology plays in *D. v. virgifera* movement into soybeans had not been investigated.

Movement of *Diabrotica virgifera* into Soybean Fields

Given the seemingly indiscriminate nature of *D. v. virgifera* oviposition, understanding how variant *D. v. virgifera* became resistant to crop rotation focused on factors that draw *D. v. virgifera* into a soybean field. Sammons et al. (1997) proposed that corn residue accumulating on the soil surface from reduced or no tillage attracted *D. v. virgifera* into soybean fields. However, olfactometer results were not consistent with this hypothesis, and later they concluded that corn residue did not attract *D. v. virgifera* into soybean fields (Sammons et al. 1997). They suggested that injury to rotated corn was due to a variant *D. v. virgifera* that preferred soybean fields as an oviposition site but did not propose a mechanism for such a preference. Spencer et al. (1999) investigated variant *D. v. virgifera* adult preference for soybeans and corn in a flight chamber. Overall, a greater percentage of females were collected on corn than soybean, even though corn tassels and silks, both sources of volatile *D. v. virgifera* attractants (Metcalf 1986), were removed prior to use in the flight chamber studies. Spencer et al. (1999) concluded that suspected variant *D. v. virgifera* do not have an attraction for soybean plants, and that a preference for soybeans is not necessary to explain movement into soybean. Rather a reduced fidelity to corn may be sufficient to explain variant *D. v. virgifera* oviposition in soybean fields.

Rationale and Significance

Diabrotica virgifera virgifera is one of the most serious pests of corn in the North Central Region of the United States (Levine and Oloumi-Sadeghi 1991). For the last 20 years, recommendations for corn rootworm management primarily involved crop rotation. Throughout the 1980's, crop rotation was considered the most effective method for preventing corn rootworm injury (Gray and Luckmann 1994, Levine and Oloumi-Sadeghi 1991). Where crop rotation was readily adopted, a significant reduction in the use of soil insecticides was achieved (Pike et al. 1991). The emergence of a crop rotation resistant *D. v. virgifera* now threatens this reduction in soil insecticide use.

The following research explores the effect of corn planting date and subsequent phenology on *D. v. virgifera* dispersal, an area of *D. v. virgifera* behavior that has not been well characterized (Levine and Oloumi-Sadeghi 1991). Our approach is to study *D. v. virgifera* physiology (adult emergence) and behavior (phenology mediated dispersal) that would affect how adult *D. v. virgifera* experience corn phenology. Though either factor could characterize variant *D. v. virgifera*, they are not mutually exclusive, and as such deserve simultaneous investigation.

Studying *D. v. virgifera* dispersal will have significant impact on our understanding of variant *D. v. virgifera* behavior, as well as benefits to rootworm management. Currently, transgenic varieties of corn resistant to *D. v. virgifera* larval feeding are projected for release in 2003. As with European corn borer (*Ostrinia nubilalis*), resistance management will be critical for the sustainable use of such a product. Unlike European corn borer, adult feeding will be an issue in managing resistance, since adult *D. v. virgifera* feed heavily on corn after emergence. O'Neal et al. (2002) demonstrated that *D. v. virgifera* prefer to feed on younger corn, and suggested host quality effects dispersal. Understanding how corn phenology influences *D. v. virgifera* dispersal will provide important insights into effective resistance management strategies.

Conceptual Model and Objectives

I suggest that the larger numbers of *D. v. virgifera* found in soybean fields than cornfields, as observed by O'Neal et al. (1999), is due primarily to dispersal from cornfields in an unattractive phenological stage. As will be discussed in Chapter 3, *D. v. virgifera* feeding on corn leaves is highly influenced by the phenological stage of the plant, and reduced acceptability of corn influences *D. v. virgifera* feeding on soybeans. In regions of extensive corn-soybean rotation, corn phenology may be responsible for the greater numbers of *D. v. virgifera* in soybeans, given that oviposition occurs coincidentally while adults visit soybean fields. I will refer to this phenomenon as the corn phenology model. A prediction from this model is that advancing corn phenology will result in increased *D. v. virgifera* dispersal. The corn phenology model suggests *D. v. virgifera* survival in a corn-soybean rotated landscape is possible without an attraction to, or preference for feeding on soybeans. This may help explain the occurrence of damage to first year corn in northeastern Iowa (Rice and Tollefson 1999), an area well outside the predicted range of the variant *D. v. virgifera* (Onstad et al. 1999).

The spread of injury to rotated corn, originating in east central Illinois, is consistent with the diffusion of a genetic change in regional *D. v. virgifera* populations (Onstad et al. 1999). I will refer to this as the genetic variation model. *Diabrotica virgifera virgifera* that oviposit in soybean will have a selective advantage because their offspring have a higher probability of survival, and thus, selection could produce a variant. Sammons et al. (1997) suggested that a variant had an increased affinity for soybeans. Spencer et al. (1999) refuted this, suggesting that survival in a corn-soybean rotated landscape did not require an attraction to soybeans, but rather decreased fidelity to corn. Combining this refined genetic variation model from Spencer et al. (1999) with the corn phenology model, dispersal from corn - mediated by corn phenology - is likely the primary behavior selected for in a corn-soybean rotated landscape. Given that corn phenology influences soybean feeding by *D. v. virgifera* from areas where corn-soybean rotation has failed to protect corn, response to corn phenology would pre-date the selection of a rotation resistant variant. Adult *D. v. virgifera* that respond to corn phenology in a corn-soybean landscape would increase wild type oviposition in soybeans as less time is spent in the aging corn. Over time, this response would select for a variant that can survive in a landscape composed of rotated corn and soybeans, resulting in a population of sufficient size to produce injury to rotated corn.

Two unique mechanisms could produced a variant whose response to corn phenology is greater than that of the wild type. The first would be a behavioral mechanism that manifests itself as an increased sensitivity to corn phenology, leading to a decreased fidelity to and greater movement out of corn. The second mechanism is physiological, with variant adult *D. v. virgifera* emerging later than the wild type. In the field, a delayed emergence would produce the same effect on movement as a decreased fidelity to corn, as later emerging individuals would encounter corn in an advanced phenological stage. This second, physiological mechanism is not mutually exclusive of the behaviorally based mechanism.

Outline of Dissertation

The goal of my doctoral research has been two-fold: assess the risk of rotation-resistant *D. v. virgifera* to Michigan corn production and account for this behavioral adaptation by testing the phenology model. In Chapter two, I present the results of a three-year survey of *D. v. virgifera* in soybean fields throughout the lower peninsula of Michigan. Coupled with this survey are measurements of adult *D. v. virgifera* emergence in cornfields that were monitored in the previous year. This emergence data provides a test of a possible physiological mechanism for the phenology model (delayed *D. v. virgifera* emergence).

The third Chapter describes a novel method for measuring herbivore damage to leaves; it was published in the Journal of Economic Entomology (O'Neal et al. 2002a). This method was developed to assist in studies of adult *D. v. virgifera* feeding on soybean leaves in Chapter four. In this chapter, the phenology model is explicitly formulated, based on the results of our various leaf feeding assays. Data presented in Chapter four was published in Environmental Entomology (O'Neal et al. 2002b). Here it is presented in an expanded form, including data from experiments employing *D. v. virgifera* raised in a lab colony.

The fifth Chapter describes the dispersal response of adult *D. v. virgifera* to corn of varying phenology. This is an attempt to characterize rotation-resistant *D. v. virgifera* by their response to corn phenology, the potential behavior mechanism of the corn phenology model. Finally, a sixth Chapter provides a brief conclusion, synthesizing the presented work for a final, take-home message.

CHAPTER 2:

MONITORING FOR ROTATION-RESISTANT *DIABROTICA VIRGIFERA VIRGIFERA* (COLEOPTERA: CHRYSOMELIDAE) IN MICHIGAN SOYBEAN FIELDS AND THE PATTERN OF SUBSEQUENT ADULT EMERGENCE IN ROTATED AND CONTINUOUS CORNFIELDS

ABSTRACT: Injury to rotated corn by *Diabrotica virgifera virgifera* Leconte has become a common occurrence across east-central Illinois and north-west Indiana. We report the results of a three-year survey (1999-2001) of soybean fields for *D. v. virgifera* in the lower peninsula of Michigan. Only two fields out of 138 had a mean number of beetles per trap per day above action thresholds (mean beetles/trap/day > 3) to suggest that injury to rotated corn can occur. To confirm that *D. v. virgifera* oviposition occurred in these soybean fields, we monitored adult emergence when these fields were planted to corn. We hypothesized that the presence of *D. v. virgifera* in soybeans may be due to a delay in emergence between rotated and continuous cornfields. Such a delay would facilitate dispersal from cornfields as adults emerging from rotated fields would encounter corn with a greater maturity. An apparent delay in adult emergence was observed in a rotated field during 2000 but was not observed in any of the rotated fields during 2001, and in only one of three fields in 2002. The highest number of *D. v. virgifera* collected occurred in southwest Michigan (Berrien County) where nearly twice as many *D. v. virgifera* emerged from rotated than continuous cornfields in southwest Michigan (Berrien County) during 2002. Counties to the northwest of Berrien (Kalamazoo, Clinton) never contributed more than 18% of the total collected for the two fields combined. We suggest that the pattern of adult emergence within these Michigan

rotated cornfields may represent the innate capacity of *D. v. virgifera* to use soybeans as an oviposition site rather than evidence for the presence of rotation-resistant beetles.

KEY WORDS: oviposition, corn, rootworms

Injury to rotated corn by *Diabrotica virgifera virgifera* Leconte is common occurrence across east-central Illinois (O'Neal et al. 2001) and north-west Indiana (Onstad et al. 1999). The current explanation for this damage (Levine and Gray 1996, O'Neal et al. 2002, Spencer et al. 1999) is that female *D. v. virgifera* move out of the cornfields from which they emerge and fly into soybean fields where they oviposit in the soil. If corn is planted the following year, the larvae which emerge can cause damage to corn roots.

O'Neal et al. (1999) tracked the immigration of *D. v. virgifera* into soybean fields with unbaited Pherocon AM yellow sticky traps in east-central Illinois. They observed large numbers (nearly 30 beetles per trap per day) of adult female *D. v. virgifera* throughout soybean fields during the month of August. Onstad et al. (1999) modeled the apparent spread this phenomenon from a point of origin in east-central Illinois. Using sweep-net data from soybeans, they suggested that movement of beetles on prevailing easterly weather systems may account for the apparent spread of injury to rotated corn from Illinois into Indiana, Ohio and Michigan. Their model further predicts the spread of rotation-resistant *D. v. virgifera* into the lower peninsula of Michigan and the western border of Ohio.

Preliminary survey efforts in Michigan found large numbers of r *D. v. virgifera* in soybean fields of southern Michigan, and documented rootworm injury to corn following soybeans, as well as alfalfa and wheat (DiFonzo 1998). Although populations of *Diabrotica barberi* with a delayed egg diapause have been reported in Michigan (Landis et al. 1998), the more recent reports of injury to rotated corn is attributed to *D. v. virgifera.*

O'Neal et al. (2001) predicted injury to rotated corn by monitoring populations of *D. v. virgifera* the prior year in soybean fields with Pherocon AM yellow sticky traps. Following the protocol of O'Neal et al. (2001), we surveyed soybean fields for *D. v. virgifera* in the lower peninsula of Michigan. To confirm that *D. v. virgifera* actually oviposited in soybean fields, we monitored adult *D. v. virgifera* emergence in the following year when that field was planted to corn.

Currently, no phenotypic or genotypic markers exist to differentiate rotation-resistant from wild type *D. v. virgifera.* O'Neal et al. (2001) suggested that rotation-resistance is due to a decrease fidelity to corn in response to the plants advancing phenology. They further hypothesized that this reduction in fidelity could have physiological basis, in that rotation-resistant *D. v. virgifera* would have a delayed emergence relative to the wild type. We tracked adult emergence to determine if a difference in the timing of *D. v. virgifera* emergence occurred between rotated and continuous cornfields.

Wild type *D. v. virgifera* are not considered to use soybean as a oviposition site (Shaw et al. 1978). Therefore, equal to greater emergence in a rotated versus its adjacent continuous cornfield may suggest the presence of rotation-resistant *D. v. virgifera.* The emergence data was also used to test this second hypothesis, by comparing ratio of total emergence from the rotated and continuous cornfields to a 1:1 ratio.

Materials and Methods

Survey of Michigan soybean fields. In the summer of 1999, we supplied volunteers (growers, scouts and extension agents) with unbaited Pherocon AM yellow sticky traps to monitor soybean fields for adult *D. v. virgifera*. Volunteers set up one trap along each of four field edges 10 m into the soybean field. Traps were supported on metal post and adjusted weekly to a height just above the soybean canopy. Traps were changed each week beginning in July through the end of August, and returned to Michigan State University for counting. Volunteers followed the same protocol in 2000 and 2001 growing seasons.

Tracking emergence: emergence cage design. We built emergence cages designed at the Illinois Natural History Survey (Fig. 2). The wooden cage has a stainless steel skirt extending 4 cm below the frame that is sunk into the soil around a corn plant. The wire-screen covered top is bisected with a cross piece that has two 6 cm diameter holes, one directly in the center and the other 10 cm from the edge. Extending from the center hole is a mesh sleeve that is held tight around the stalk of the plant with a twist-tie. Once placed around the stalk when the corn is approximately 0.5 m tall, the corn plant is able to continue growing unimpeded by the cage. Emerging beetles are collected through a funnel placed over the second hole and covered with a 5.5 cm diameter glass jar.

Tracking emergence: 2000. In the 1999 survey, sticky trap catches in a single soybean field in Clinton County was over the threshold proposed by O'Neal et al (2000, 3 beetles/trap/day). Rootworm emergence in this field was monitored in 2000 when the

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field was rotated to corn. An adjacent continuous cornfield (planted to corn for 5 years) was monitored as a comparison. Both fields were treated at planting with chlorpyrifos (Lorsban, Dow AgroSci, 3.6 kg per acre) and managed using conventional agronomic practices by the grower cooperator. On 12 July, twenty emergence cages were distributed across four rows in each field. The rows were approximately 12 m apart; cages within row were separated by 66 m from each other and the edge of the field. Cages were checked once a week and all contents removed.

Tracking emergence: 2001. Results from 2000 suggested that the timing of adult emergence in rotated fields differed from continuous cornfields. In 2001 we monitored adult emergence, to test if the timing of adult emergence differed between rotated and continuous cornfields. Since the survey results from 2000 did not reveal any rotated cornfields at risk for *D. v. virgifera* injury, we sought assistance from extension agents in selecting field sites. We selected three pairs of fields, a rotated and a continuous cornfield no less than 50 m apart. One pair of fields was located in St. Joseph County, Indiana, where injury to rotated corn was reported. Two additional sites in Kalamazoo County, Michigan, did not have a history of injury to rotated corn. We used 20 emergence cages per cornfield. Cages were arranged in same manner as in 2000, sealed on 6 July and checked every three to four days. None of the fields were treated with a soil insecticide.

Tracking emergence: 2002. From the 2001 survey, sticky trap catches in two soybean fields in Berrien County were over the threshold proposed by O'Neal et al (2000). In 2002, we selected these two fields to track emergence. The rotated fields in Berrien County were planted without a soil insecticide. Only a single continuous cornfield was found as a comparison for both rotated cornfields, at a distance of ~1400 m from both fields. An additional continuous cornfield could not be located in 2002.

In 2002, we monitored emergence in Clinton County in the same field that was monitored in 2000. The rotated cornfield in Clinton County was treated with imidacloprid, 1.34 mg a.i. (Gaucho™, Bayer) / kernal seed treatment, but no soil insecticide. The continuous cornfield was within 100m of the continuous cornfield monitored in 2001 and planted with chlorpyrifos at 3.6 kg a.i. per acre. In both Clinton and Berrien County, we used 20 emergence cages per cornfield. All cages were sealed by 8 July.

Experimental Design and Data Analysis. To observe if *D. v. virgifera* emergence is delayed in rotated versus continuous cornfields, we report the timing of emergence for each field pairs. The timing of emergence is reported as a proportion computed from the cumulative emerged divided by the total collected. For the second hypothesis, the ratio of total emergence between the rotated and continuous cornfields was calculated. At each site, the ratio of emergence in the rotated and continuous cornfields was compared to a 1:1 ratio with a Chi-square goodness of fit test (X^2, Sokal and Rohlf 1995).

Results

Survey of Michigan soybean fields. During the three years of our survey, we tracked *D. v. virgifera* in 138 fields in 26 counties (Table 1). Only seven out of twenty six counties had a mean higher than one beetle per trap per day. Except for Clinton County, the remaining five counties that averaged more than one beetle per trap per day are within the first two tiers of counties bordering Illinois and Indiana. Only three fields, one in Clinton

County in 1999 (Fig. 2a) and two in Berrien County in 2001 (Fig. 2b), had a mean number of beetles per trap per day high enough to suggest that injury to rotated corn may occur (O'Neal et al. 2000). In general, the number of beetles and timing of their immigration into the soybean field was very similar for these two fields (Fig. 2a and b).

Tracking emergence: 2000. All beetles collected in the rotated and continuous cornfields at the Clinton County site were *D. v. virgifera*, and many still teneral when they were removed from the emergence cages. We collected a total of 35 adult *D. v. virgifera* in the rotated cornfield in Clinton County (Table 2), and 178 were collected in the adjacent continuous cornfield. . The timing of emergence between the rotated and continuous cornfields appeared to differ (Fig. 3), with the time to 50% cumulative emergence occurring later (ca. 7 days) in the rotated cornfield.

Tracking emergence: 2001. At both Michigan sites and the Indiana site we collected more adult *D. v. virgifera* in the continuous than rotated cornfield. We collected the greatest number of *D. v. virgifera* (63) from a rotated cornfield at our Indiana site in 2001 (Table 2). However, the greatest total (both rotated and continuous combined) number of *D. v. virgifera* collected was from our Climax field site in Kalamazoo County, Michigan (Table 2).

For the site in Indiana, the numbers of beetles emerging in the rotated and continuous cornfields was statistically indistinguishable from a ratio of 1:1 ($P = 0.16$; Table 2). In all cases the Michigan sites had a ratio of emergence between the rotated and continuous cornfields that was significantly different from an expected ratio of 1:1 (Table 2). The rotated fields in Michigan never contributed more than 16% of the total beetles collected for the two fields combined.

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In 2001, we did not observe a delay in the emergence of adult *D. v. virgifera* from the rotated cornfields (Fig. 4). The earliest adult emergence (as measured by the date to 50% cumulative emergence) was observed in the Indiana rotated cornfield (Fig. 4a). Where we saw emergence from a rotated field in Michigan (Fig. 4b), the date of 50% cumulative emergence occurred at the same time as that of the continuous cornfield.

Tracking emergence: 2002. In 2002, the highest number of *D. v. virgifera* collected during the three year study occurred in southwest Michigan (Berrien County, Table 2) where nearly twice as many *D. v. virgifera* emerged from rotated than continuous cornfields in southwest Michigan. Though large numbers of beetles were collected from the rotated fields, there emergence was not delayed in comparison to the continuous cornfield (Fig. 5a).

Both the number and timing of emergence of *D. v. virgifera* at Clinton County during 2002 was similar to that of 2000. In both years, a lower number of beetles emerged from the rotated field, and again , there was an apparent delay in the emergence of beetles from the rotated cornfield.

Discussion

Only three Michigan soybean fields (Table 1; one in Clinton County in 1999 and two in Berrien County in 2001) had sufficient numbers of *D. v. virgifera* adults were collected to suggest injury to rotated corn was probable. The profile of trap capture over time (Fig. 2 a and b) was similar to those observed by O'Neal et al. (1999) for *D. v. virgifera* in soybean fields of east-central Illinois, were rootworm injury to rotated corn is common. In other Michigan soybean fields, *D. v. virgifera* adults were collected in very low r

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low numbers (1 to 5 per trap) and often only during one to two weeks of the eight-week sampling period.

Surprisingly, in 1999, the only Michigan soybean field of the ones surveyed to be at risk for injury in 2000 was in Clinton County, far north of the Illinois border. In 2000, we recovered adult *D. v. virgifera* when that field was rotated to corn. Many of the beetles collected in 2000 and 2001 were teneral when removed from the emergence cages, and a prolonged (more than one year) diapause has not been observed in *D. v. virgifera* (Landis et al. 1992). Therefore, we suggest that the adults emerging from the rotated cornfields in 2000 and 2001 are due to oviposition in the previous year.

Sampling soil for *D. v. virgifera* eggs in rotated corn and soybean in east-central Illinois suggest that both are used equally as oviposition sites (C. Pierce and M. Gray, personal communication). Of the pairs of rotated and continuous cornfields monitored for *D. v. virgifera* emergence (Table 2), those in South Bend, Indiana (2001) and Berrien County, Michigan are more likely to have rotation-resistant individuals, given that the ratio of emergence between these two fields is 1:1, or exceeded a 1:1 ratio in favor of rotated corn.

The apparent delay in adult emergence observed in the rotated field during 2000 (Fig. 3) was not observed in any of the rotated fields during 2001 (Fig. 4, a and b), or in 2002 (Fig 5b). In 2002, curiously, we did observe a delay in emergence from the rotated field in Clinton County (Fig 5a). O'Neal et al. (2002, Chapter 3) have suggested a delay in adult *D. v. virgifera* emergence from rotated corn as a possible mechanism for rotation-resistant *D. v. virgifera*. Based on the data presented here, a delay in adult emergence is not likely, as it was only observed in one site (Clinton County). It may be that the beetles that

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that emerged in the other rotated fields represent a mixture of wild-type and 'rotation-resistant'. Without a morphological or molecular marker to separate rotation-resistant *D. v. virgifera* from the wild type, we cannot attribute the adults emerging from rotated corn in Michigan to this variety or to some base-level oviposition by the wild-type in soybean fields. Since the number of beetles that emerged from the rotated field in Clinton and Kalamazoo Counties is so small in relation to the number that emerged from the adjacent continuous cornfields, it is unlikely that these populations are 'rotation-resistant'. This suggests that the low level of beetles we collected in these counties represents the latent ability of *D. v. virgifera* to use soybeans as an oviposition site.

Table 1. Mean (beetles/trap/day/field) numbers of *D. v. virgifera* in Michigan soybean fields

County	County mean[a] (number of fields sampled)		
	1999	2000	2001
Allegan	0.46 (1)	NA	NA
Arenac	NA	0.0 (4)	NA
Barry	0.24 (4)	NA	NA
Bay	0.0 (2)	NA	NA
Berrien	1.1 (5)	0.22 (4)	1.95 (4*)
Branch	0.59 (4)	NA	NA
Calhoun	0.42 (3)	0.05 (1)	0.17 (3)
Cass	1.03 (6)	0.30 (7)	1.18 (3)
Clinton	1.82 (3*)	0.21 (1)	1.04 (1)
Eaton	0.23 (3)	NA	NA
Hillsdale	0.57 (3)	NA	NA
Huron	0.22 (5)	NA	NA
Ingham	0.22 (1)	NA	NA
Ionia	0.64 (1)	0 (1)	NA
Isabella	0.62 (1)	0.17 (1)	0.09 (1)
Kalamazoo	0.60 (3)	0.05 (2)	0.46 (3)
Lapeer	NA	0.01 (2)	0.09 (2)
Lenawee	0.36 (3)	0.07 (2)	0.05 (3)
Livingston	0.17 (3)	NA	NA

Monroe	0.07 (3)	0.12 (4)	0.01 (5)
Saginaw	0.0 (3)	NA	0.02 (1)
St. Joseph	0.21 (7)	0.13 (4)	0.24 (1)
Sanilac	0.65 (4)	NA	NA
Shiawassee	0.07 (1)	NA	NA
Tuscola	0.29 (1)	NA	NA
Washtenaw	1.35 (1)	NA	NA
Total fields monitored	71	40	27

[a] Mean is calculated from the mean of each field. Field means calculated from a four week period starting on the last week of July to the third week of August.

* Indicates County contained an individual field that had a mean beetle/trap/day above the action threshold

Table 2. Total number of *D. v. virgifera* collected in emergence cages in adjacent continuous and rotated cornfields

Site	Year	Field type[a]	Sum[b]	Percent[c]	X^2	P[d]
Clinton Co. Michigan	2000	Continuous	178	84		
		Rotated	35	16	96	P < 0.01
South Bend, Indiana	2001	Continuous	84	57		
		Rotated	63	43	3	$P = 0.16$
Kalamazoo Co. Michigan	2001	Continuous	9	100		
(Mendon)		Rotated	0	0	9	$P < 0.01$
Kalamazoo Co. Michigan	2001	Continuous	247	92		
(Climax)		Rotated	22	8	188	$P < 0.01$
Clinton Co. Michigan	2002	Continuous	165	82		
		Rotated	35	18	84.5	P < 0.01
Berrien Co. Michigan	2002	Continuous	264	40 (28)[e]		
		(Rotated field #1)	391	60	114	P < 0.01
		(Rotated field #2)	664	72	306	P < 0.01

[a]Continuous cornfields were fields that were planted to corn for a minimum of two years, rotated cornfields were planted to soybeans in the previous year.

[b] The sum is calculated from the total number of beetles collected in the cages during the sampling period. Twenty emergence cages per field were used in 2001 and 40 cages per field were used in 2000.

[c] The percentage is calculated based on the contribution of each field to the total number of beetles collected in the two adjacent fields.

[d] P value is for X^2 comparison of the percent contribution of each field to a 1:1 ratio.

[e] Berrien Rotated field #2 was used to a calculate the percentage and subsequent X^2 value

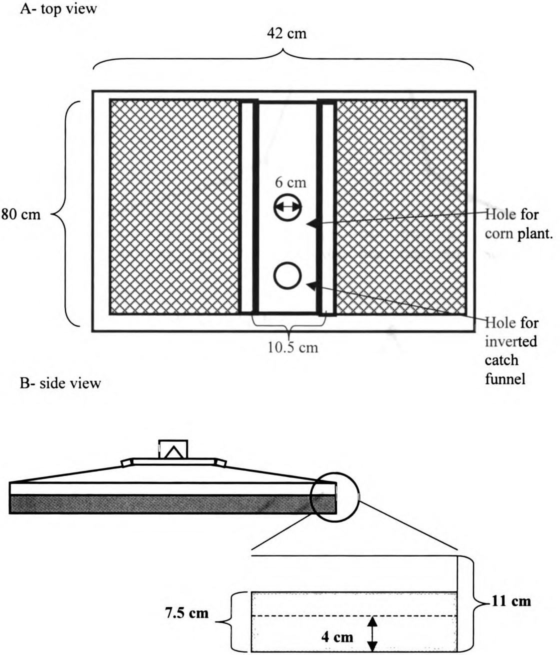

Fig. 1. Diagram of emergence cage for adult *D. v. virgifera*. The cage is placed such that the center hole of the cage fits around the corn plant (A, top view). The metal skirt of the cage (B, side view) is pushed into the soil.

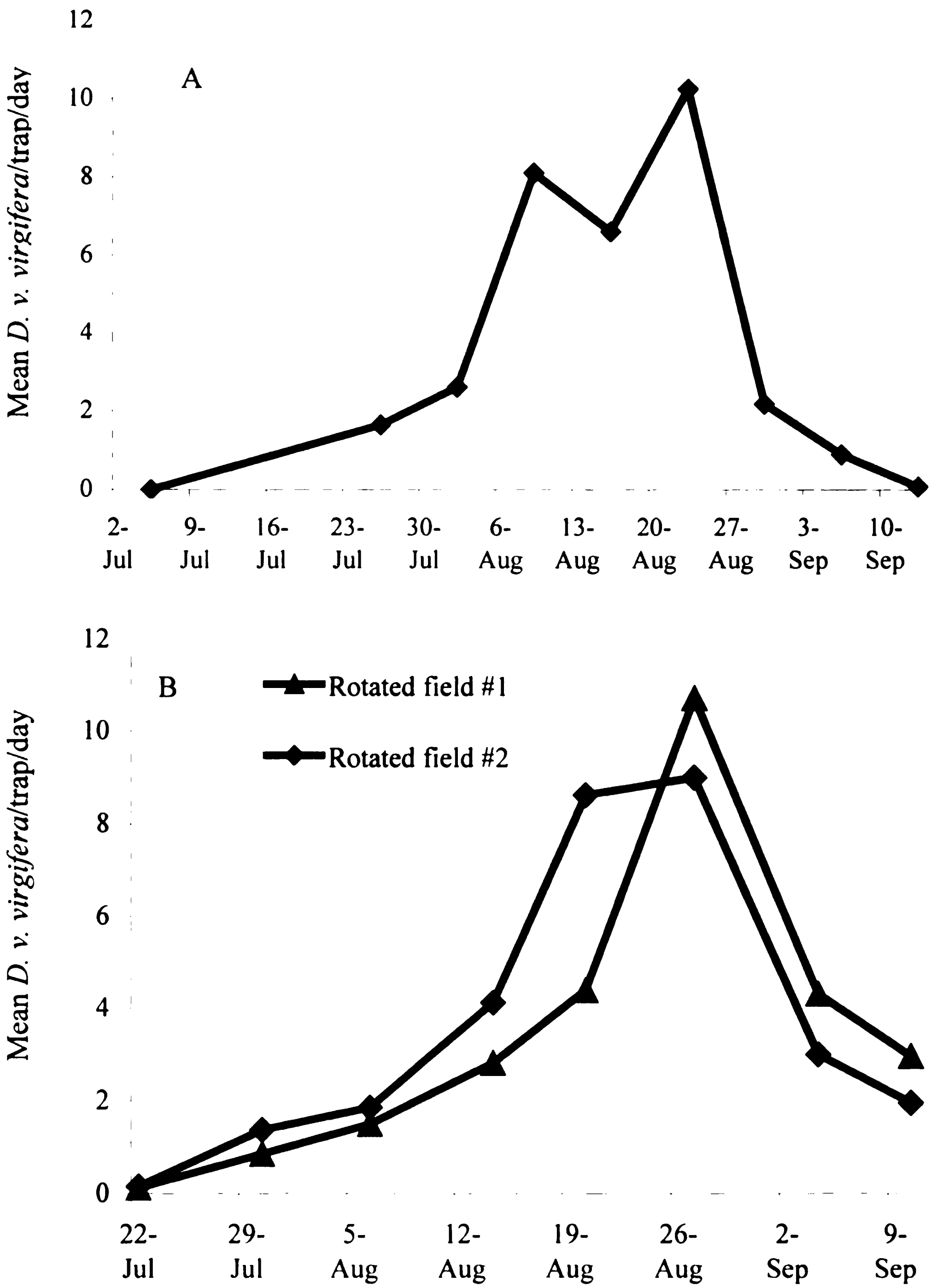

Fig. 2. Mean number of beetles per trap per day collected in a soybean field of (A) Clinton County, Michigan during the 1999 growing season and two soybean fields in (B) Berrien County during the 2001 growing season.

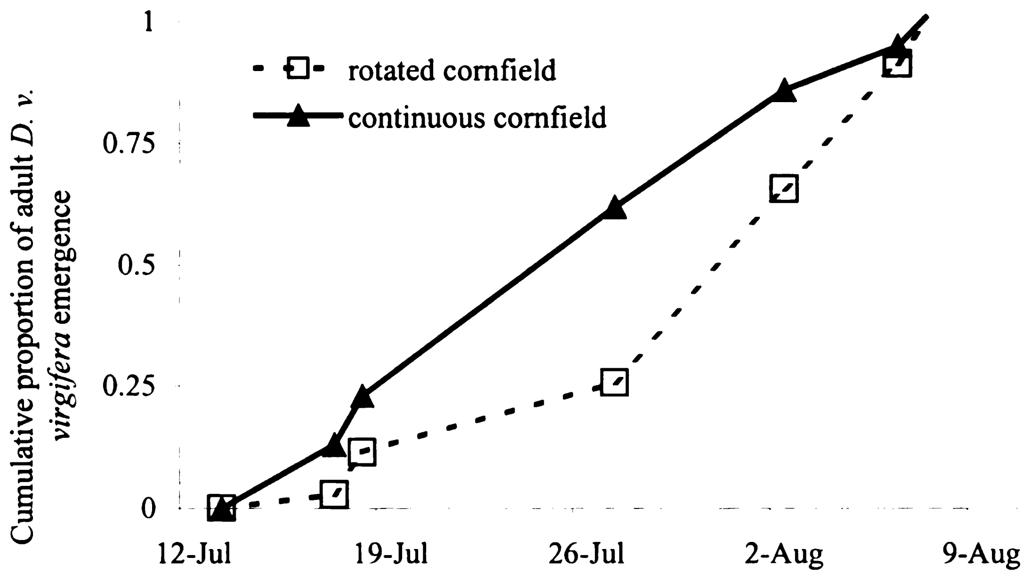

Fig. 3. Cumulative proportion of adult *D. v. virgifera* emergence from adjacent rotated and continuous cornfield in Clinton County during the 2000-growing season. Proportion is calculated from the total collected within each individual field.

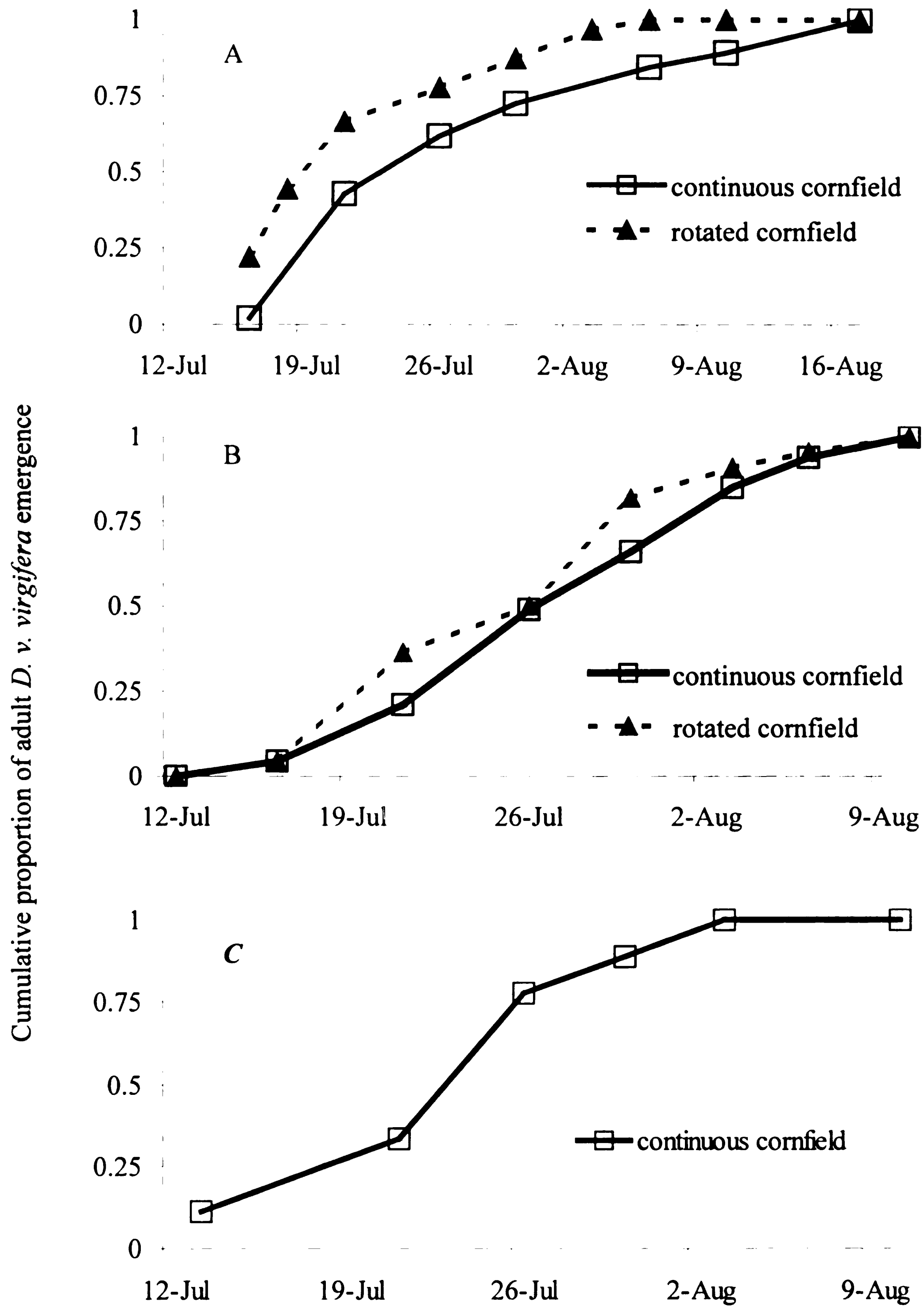

Fig. 4. Cumulative proportion of adult *D. v. virgifera* emergence from adjacent rotated and continuous cornfield near (A) South Bend, Indiana and in Kalamazoo County in Michigan near (B) Climax and (C) Mendon during the 2001 growing season.

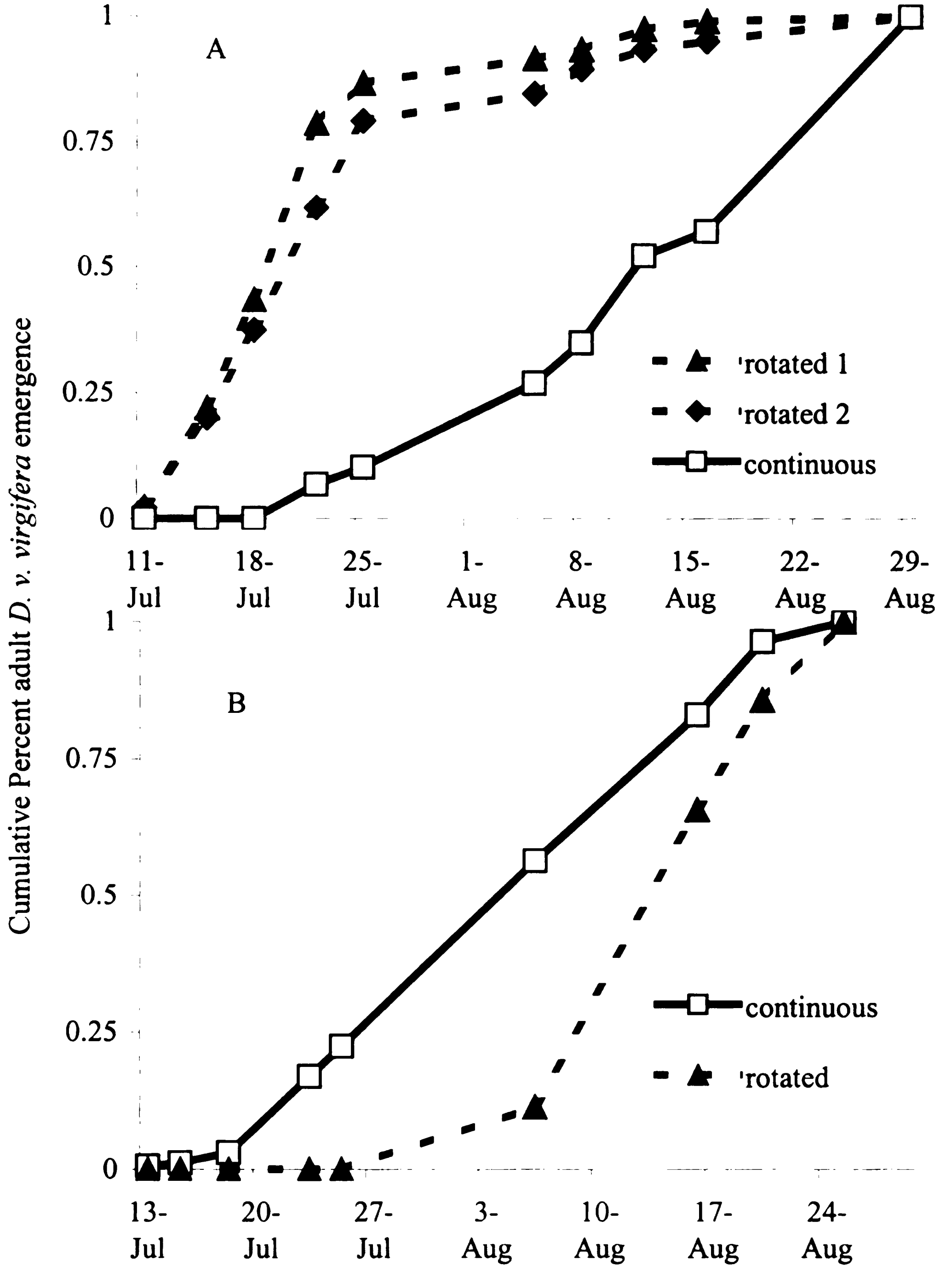

Fig. 5. Percentage of the cumulative adult *D. v. virgifera* emergence from adjacent rotated and continuous cornfield in and (A) two rotated and one continuous cornfield in Berrien County (B) Clinton County during the 2002 growing season.

CHAPTER 3:

AN INEXPENSIVE, ACCURATE METHOD FOR MEASURING LEAF AREA AND DEFOLIATION THROUGH DIGITAL IMAGE ANALYSIS

ABSTRACT We report a protocol using a common desk-top scanner and public domain software for measuring existing leaf area and leaf area removed as a result of herbivory. We compared the accuracy and precision of this method to that of a standard leaf area meter. Both methods were used to measure metal disks of a known area, the area of soybean (*Glycine max*) leaves, and the area removed by simulating leaf feeding with a hole-punch. We varied the amount of injury across a low, medium, and high degree of simulated feeding. The mean area of 10-cm^2 and 50-cm^2 metal disks was more accurately estimated with the leaf area meter than the desk-top scanner. Leaf area estimates from both methods were highly correlated. The desk-top scanner estimated the leaf area removed from the low, medium or high degree of simulated leaf feeding. However, the leaf area meter overestimated low levels of simulated feeding injury. Though measuring a leaf's surface area with a desk-top scanner requires two steps (creating a digital image file and calculating the area represented by that image), the overall time required to measure leaf injury is shorter than with a leaf area meter. This relatively simple and inexpensive method of estimating leaf area and feeding damage has advantages in certain experimental situations where a pre-feeding measurement of the leaf is impossible or undesirable, or when small amounts of feeding occur.

KEY WORDS leaf area, herbivory, video image analysis, soybean

Many biological studies require measurement of leaf area and defoliation. Measuring leaf area removed as a result of insect herbivory can be useful for evaluating host plant resistance (Jansky et al. 1999), pesticide activity (Gonzalez et al. 1992, Hoy and Hall 1993, Wheeler and Isman 2001), and plant-insect interactions (Peterson et al. 1993, Hammond et al. 2000). Prior studies measuring herbivory (leaf area removed) have used visual estimates (Stotz et al. 2000), hand tracings of injured leaves (Hoy and Hall 1993), or a comparison of treated leaves to an appropriate control (Gonzalez et al. 1992, Wheeler and Isman 2001). The latter methods use a leaf area meter or a sampling grid to estimate leaf area. Though useful, each of these methods has significant drawbacks; leaf area meters are expensive and measuring leaf area by hand with a sampling grid is time consuming.

Recently, digital cameras have been used to measure infection by plant pathogens (Lindow and Webb 1983), insect feeding (Alchanatis et al. 2000, Su and Messenger 2000), and to capture insect images (Mitchell and Laswell 2000). James and Newcombe (2000) used the Adobe Photoshop (version 3.0, Mountain View, CA) software package to measure leaf feeding of *Phratora califonrica* (Coleoptera: Chrysomelidae) on hybrid poplars (*Populus* spp.). These estimates of leaf feeding were used to train personnel to visually estimate percentage of damage in leaf samples. Using public domain software (Scion Image) and a digital imaging system, Wheeler and Isman (2001) measured area of untreated and treated leaf discs. We have developed a related protocol using a common desk-top scanner and public-domain software to measure existing leaf area and area removed by herbivory. In this study, we validated this method by comparing it to a standard leaf area meter.

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Materials and Methods

Creating a digital image of a leaf. Individual leaves or objects of known area were scanned into a digital format using a Hewlett Packard ScanJet 6200C desk-top scanner (Hewlett Packard, Cupertino, CA) using HP Precision Scan Pro (version 1.1, images scanned at 150 dpi) software. Both the scanner and the software were operating on a Dell Optiplex GX1 computer with a Pentium III processor with 128 MB RAM. An object was placed on the scanner; the lid of the scanner was closed, and a preliminary scan was made using the preview feature of the software. The preliminary image was converted from color to grayscale (selected from the output type menu). The highlight and shadow levels within the exposure adjustment (selected from the tools menu) were manipulated to create a black image on a white background. The final version was saved as a TIFF file without LZW compression. Formats that involve compression (GIF, JPEG, and TIFF with LZW compression) are not compatible with the image analysis software.

We used public domain software (Image 4.0.2 for Windows, National Institute of Health, Bethesda, MD) to measure the surface area of objects in a digital format. This program is available for both Apple and Windows operating systems, and as of 14 May, 2002, could be downloaded at the following addresses: Apple (http://rsb.info.nih.gov/nih-image/) and Windows (http://www.scioncorp.com). We opened the TIFF file to be analyzed within the Image software and using the set scale option (selected from the analyze menu), selected a unit (cm) to convert pixels to a unit of measurement. We included a standard of known dimensions (a ruler) within the image for calibrating the pixel conversion. Once the units had been selected, the grayscale-image was adjusted so that the image was composed of only black and white. Within the map box (selected

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from the Windows menu), a threshold option was selected to convert all color values to either a one (black) or zero (white). The image could then be selected with the magic wand (selected from the tool box menu). A black region was selected with the magic wand. The software moved to the right of the spot until the margin of the region was reached and the entire contiguous region was outlined. With the object outlined, the surface area was calculated by selecting the measure option (selected from the analyze menu).

We also measured the amount of leaf area removed. While the leaf was selected, the damaged area was highlighted after inverting the colors of the image (inverse selected from the edit menu). The amount of leaf area removed was measured from this inverted image within the boundaries of the original selection.

Experimental design and data analysis. We compared the accuracy and precision of leaf area estimates from the desk-top scanner with estimates from a LI-COR LI-3000 (LI-COR, Lincoln, NE) leaf area meter. Three separate tests were conducted using metal disks of a known area, a single soybean leaf, and multiple soybean leaves.

In our first test, metal disks were used to evaluate the precision and accuracy of both methods to estimate a known area (10 cm^2 or 50 cm^2 disk). Each disk was scanned five separate times with both the leaf area meter and the desk-top scanner. The location of the disk on the desk-top scanner and leaf area meter was adjusted with each scan. Descriptive statistics were used to compare the accuracy (mean) and precision (standard error of the mean, SEM) of each method. We used Student's t-test to determine if the areas estimated by either method differed from the actual area of the metal disks.

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In our second test we compared the leaf area meter with the desk-top scanner to estimate leaf area and leaf area removed from a single soybean leaf. A single soybean (*Glycine max* L., 'Asgrow Ag2201') leaf was harvested and the petiole removed from a plant in the late reproductive stage grown in a greenhouse. The middle leaflet was taken from the third most distal trifoliate. The uninjured leaf was damaged with 1, 5, 20 and eventually 40 non-overlapping holes (0.13 cm^2) from a metal hole-punch. All holes were made within the boundaries of the leaf. At each level of injury (including the uninjured condition), the leaf was scanned five separate times, with its position on the desk-top scanner and leaf area meter adjusted with each scan.

In a third test, we used multiple soybean leaves that ranged in size as a more practical test of the accuracy of the desk-top scanner to measure leaf and hole area. Unlike the previous test, only a single scan was made with the desk-top scanner. However, five separate scans were made with the leaf area meter. Leaves that had a minimal amount of pre-existing damage were randomly assigned to four treatment groups of five leaves each: no damage, 1 hole per leaf, 5 holes per leaf, or 25 holes per leaf, and leaves were damaged as before.

In each test, the means and SEM of leaf area and leaf area removed were calculated. In the second test, because the true area of the individual leaf was not known, we used paired *t*-tests to determine if the difference between the leaf area estimated from both methods differed from zero. Student's *t*-tests analyzed if the difference between the estimated leaf area removed by either method differed from the actual area removed by the hole punch. In the second test, we also conducted a correlation analysis on the leaf area as measured by both methods (SAS 1996).

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Time required to measure leaf and damage area. We measured the total time to scan a single leaf five-times with the leaf area meter, per the method described above. Five different leaves were measured in this way and a mean time calculated. For the desk-top scanner, we measured the time to convert five sets of images with five leaves each to a single digital image with the desk-top scanner. We then measured the time to calculate leaf area and injury (leaf area removed) from these digital files. All time estimates assumed that a user was familiar with the software and hardware and that the hardware was on long enough to begin working immediately ('warmed-up').

Results

The mean surface area of a 10-cm^2 and 50-cm^2 metal disk was more closely estimated with the leaf area meter than the desk-top scanner. For both disks, the mean surface area estimated by the leaf area meter was not significantly different from the actual surface area (Table 1). The surface area estimated by the desk-top scanner was significantly different from the actual area of both disks. The desk-top scanner slightly overestimated the surface area.

Neither the leaf area meter nor the desk-top scanner consistently overestimated the area of the single soybean leaf (Table 2), when compared to each other. Only the estimated leaf area of the damaged leaf with 5 holes was clearly similar for both methods. Though there may have been a marginal difference ($P = 0.07$) between the methods when the leaf was damaged with one hole, all other estimates of this leaf's area, undamaged or damaged, were significantly different between the two methods. In general, where significant differences occurred, estimates made from the leaf area meter were greater than those of the desk-top scanner. Estimates of leaf area from the desk-top scanner were generally less variable than estimates from the leaf area meter. For this individual leaf measured multiple times, the desk-top scanner more closely estimated the actual leaf area removed while the leaf area meter consistently overestimated the area removed (Table 3).

Leaf area estimates from both methods were significantly correlated (Fig. 1, n = 20, $r = 0.99$, df = 1, $P < 0.0001$) for leaves ranging in size from 8.11 cm^2 to 76.42 cm^2. No significant divergence from this pattern was evident at any leaf size. When estimating a constant level of simulated herbivory to multiple leaves, the leaf area meter's estimate of herbivory simulated by a single hole was significantly greater than the actual value (Table 4, $t = 6.09$, df = 1, $P = 0.004$). Unlike the previous test, where a single leaf was scanned multiple times with the desk-top scanner and a mean area calculated, in this test many leaves were scanned only once. As in the first test, the one-hole level of injury was accurately estimated by the desk-top scanner. Also, the desk-top scanner accurately estimated the simulated herbivory from five and 25 holes. The SEM of measurements taken with the leaf area meter was consistently higher than those of the desk-top scanner.

The average time to measure the surface area and injury area of a set of five leaves with the desk-top scanner and Image software was 3:25 $\pm$ 0:03 (min:sec), which included creation of the digital file and measuring each leaf within that file. To measure the area of those same five soybean leaves using the leaf area meter, the average time was 6:10 $\pm$ 0:10, almost twice the time needed with the desk-top scanner.

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Discussion

Measuring the surface area of a metal disk, the leaf area meter was more accurate and had greater precision then the desk-top scanner (Table 1). Measuring the area of a single leaf, we did not observe a consistent trend of one method overestimating area relative to the other (Table 2). We observed greater precision in measurements made with the desk-top scanner, as seen in the consistently lower SEM values than those from the leaf area meter. A high degree of correlation existed between the two methods in estimating leaf area (Fig. 1). So, although our first test indicated superior performance of the leaf area meter, the desk-top scanner performed as well if not better in the latter two tests involving single and multiple soybean leaves. Two differences in the construction of the desk-top scanner versus the leaf area meter may be responsible for the difference in performance between a hard, flat object like the metal disks and a leaf with varying thickness. The desk-top scanner's cover may provide better horizontal compression. Keeping the leaf static and moving the scanning optics, unlike the leaf area meter that moves the object across a static optic, may also assist measuring leaf area as well as estimating herbivory.

The desk-top scanner performance surpassed that of the leaf area meter in estimating simulated herbivory. In our second test with increasing levels of injury to a single leaf, the leaf area meter was unable to accurately estimate the amount of leaf area removed (Table 3). However, the desk-top scanner accurately estimated the amount of leaf area removed in two of the four levels of simulated herbivory. In the final test (Table 4), the leaf area meter was unable to accurately estimate the injury produced by a single hole

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hole punch (0.13 cm^2). The leaf area meter was able to accurately estimate the 0.65 cm^2 and 3.25 cm^2 level of injury (Table 4). However, the precision of these measurements made by the leaf area meter was less than that of the desk-top scanner.

Improvement in any given methodology often involves selecting from two of the three following attributes; quality, expediency, and cost. We have demonstrated that a desk-top scanner can accurately and precisely measure leaf area and area removed by herbivory. We have also shown that this method is faster and possibly easier to use than a leaf area meter when the goal is measuring defoliation. To measure defoliation with a leaf area meter, the leaf area before herbivory must be estimated. Given certain experimental protocols and depending upon the feeding behavior of the insect, this is not always possible or convenient. However, the desk-top scanner method does not require such an initial measurement. Also, the 20 cm by 30 cm dimension of the desk-top scanner allows for multiple leaves to be scanned. More than one leaf can be included in the digital image file, further saving time when area measurements are made.

Difficulties in accurate measurement of feeding can arise when feeding occurs at the leaf edge and the magic wand selection tool cannot follow the original leaf margin. By connecting the edges of this break with the line drawing tool, an estimate can still be made, though it may underestimate total injury. To avoid this potential disadvantage of using the desk-top scanner, we have modified our feeding assays to prevent such an underestimate by presenting test subjects with leaf tissue of a square shape (O'Neal et al. 2002). Using leaves cut to this shape allowed us to connect edges disrupted by leaf feeding. We should also point out that the leaf feeding measured by O'Neal et al. (2002) did not involve the complete consumption of the entire tissue at a feeding site. By adjus

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adjusting the exposure of the scanner, we were able to distinguish the areas where feeding occurred. The last issue of cost is much clearer. Desk-top and portable leaf area meters range in cost from $2000 to $3000, respectively. Though prices can vary, a desk-top scanner (HP ScanJet 5300c) typically costs $200-$300 or less.

Digital image analysis has been used in a variety of novel methods for applied agricultural research. A more complex methodology for measuring insect feeding was developed by Alchanatis et al. (2000) using a video-image system. Their methodology allowed for real time measures of feeding behavior by coupling digital video images with a personal computer to measure the amount and timing of leaf feeding.

A closely related technique to that presented here was developed by Su and Messenger (2000), who used digitized images to estimated termite consumption of wood. Su and Messenger (2000) were able to measure twice the area of the HP Scanjet 6200C by using a set focal distance with a digital camera. They converted these pictures to a black and white digital image before measuring the surface area (SigmaScan 2.0, Jandel Corporation 1995) and observed a significant relationship between wood weight loss and surface area removed. Though not explored here, the protocol of Su and Messenger (2000) suggests that leaf area and defoliation could be measured in a non-destructive manner by replacing the desk-top scanner with a digital camera.

Table 1. Mean surface area $\pm$ SEM of standard metal disks as measured by two methods

	Leaf area meter			Desk-top scanner		
Disk area, cm^2	Area, cm^2	t	P	Area, cm^2	t	P
10	10.04 ± 0.02	1.65	0.17	10.17 ± 0.02	7.61	0.002
50	50.05 ± 0.04	1.26	0.28	50.33 ± 0.08	4.00	0.02

Student's t-tests compared difference of estimated disk area to actual disk area (df = 4) for each method.

Table 2. Mean surface area (cm^2) $\pm$ SEM of a single soybean leaf before and after simulated herbivory as measured by two methods

Object	Leaf area meter	Desk-top scanner	t[a]	P
Undamaged leaf	29.74 $\pm$ 0.02	29.54 $\pm$ 0.01	9.00	0.0008
Damaged leaf				
1 hole	29.36 $\pm$ 0.02	29.41 $\pm$ 0.01	2.44	0.07
5 holes	28.54 $\pm$ 0.02	28.54 $\pm$ 0.02	0.21	0.84
20 holes	25.82 $\pm$ 0.03	25.61 $\pm$ 0.02	9.95	0.0006
40 holes	22.84 $\pm$ 0.03	22.31 $\pm$ 0.04	13.00	0.0002

n = 5 measurements of the same leaf

[a] Paired t-tests compared difference of estimated leaf area of each method to zero (df = 1).

Table 3. Mean area (± SEM) removed from a single soybean leaf by simulated herbivory as measured by two methods

Damaged leaf	Leaf area removed (cm^2)	Leaf area meter			Desk-top scanner		
		Area, cm^2	t[a]	P	Area, cm^2	t[a]	P
1 hole	0.13	0.38 ± 0.02	14.3	0.0001	0.13 ± 0.00	0.0	1.0
5 holes	0.65	1.20 ± 0.03	36.3	<0.0001	0.67 ± 0.002	6.5	0.003
20 holes	2.60	3.92 ± 0.03	50.3	<0.0001	2.62 ± 0.007	3.5	0.02
40 holes	5.20	6.90 ± 0.03	67.6	<0.0001	5.20 ± 0.01	0.0	1.0

n = 5 measurements of the same leaf

[b] Student t-tests compared difference of estimated hole area to actual hole area (df = 4) for each method

Table 4. Mean area removed (± SEM) from multiple soybean leaves by simulated herbivory as measured by two methods

Number of holes	Leaf area removed, cm^2	Area cm^2 Leaf area meter	Desk-top scanner	Mean difference cm^2
1	0.13	0.29 ± 0.03^{a}	0.16 ± 0.02^{ns}	0.13 ± 0.05^{b}
5	0.65	0.77 ± 0.07^{ns}	0.67 ± 0.01^{ns}	0.09 ± 0.07^{ns}
25	3.25	4.82 ± 1.24^{ns}	3.15 ± 0.09^{ns}	1.67 ± 1.24^{ns}

n = 5 leaves

[a] Paired *t*-test between actual and estimated leaf area (t =6.09, df = 1; P = 0.004)

[b] Paired *t*-test between estimated hole-area from both methods (t = 2.68, df = 1; P = 0.06)

Leaf area measured with a leaf area meter (cm^2)

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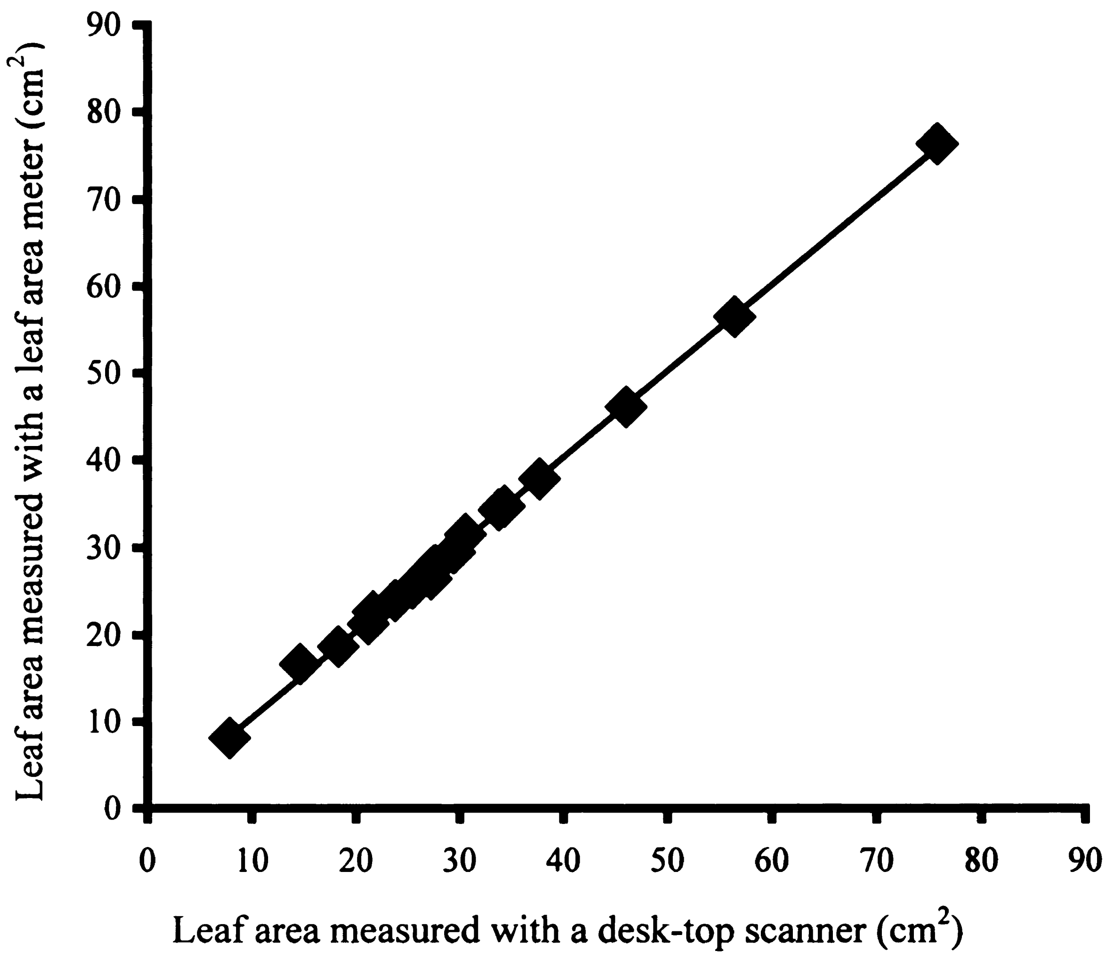

Fig. 1. Relationship between leaf area (cm^2) measured with a LI-COR 3000 leaf area meter compared with leaf area measured from a digital image created with a Hewlett Packard ScanJet 6200C desk-top scanner and analyzed using the NIH Image software. The line drawn represents the linear regression ($y = 1.0x + 0.5$, $R^2 = 0.99$, df = 1, 19; $P < 0.001$).

CHAPTER 4:

DIABROTICA VIRGIFERA VIRGIFERA (COLEOPTERA: CHRYSOMELIDAE) FEEDING ON CORN AND SOYBEAN LEAVES IS INFLUENCED BY CORN PHENOLOGY

ABSTRACT

The failure of crop rotation to protect corn from larval western corn rootworm (*Diabrotica virgifera virgifera* LeConte) injury has become common in regions of Illinois and Indiana, and is apparently spreading east into Ohio and Michigan. The extensive use of a corn-soybean rotation is considered to have selected a variant of the western corn rootworm that has expanded its ovipositional range to include soybean fields. Laboratory and field observations suggest that suspected variant western corn rootworm adults have a greater acceptance for soybean foliage as an adult feeding site than that of wild type adults. We attempted to identify variant western corn rootworm populations based on their propensity to feed on soybean foliage and what factors influence the consumption of soybean foliage. Feeding on soybean and corn leaves was quantified in laboratory feeding assays. There was no significant difference in amount of soybean leaf area eaten by western corn rootworm from Illinois versus those from Nebraska or Michigan, both regions were rotation failures have not been reported. To identify what factors influence *D. v. virgifera* feeding on soybean, we first demonstrated that western corn rootworm feeding on corn foliage was influenced by corn phenology. Corn phenology was also observed to influence the consumption of soybean leaves; more soybean leaf area was consumed in the presence of corn leaves from reproductive stage corn than younger corn

leaves. A phenology effect was observed also with corn silks, though soybean consumption was reduced in the presence of corn silks compared with leaves. Given that western corn rootworm acceptance for soybean increases in the presence of older corn, we propose an explanation for western corn rootworm oviposition in soybeans based on corn phenology.

KEY WORDS oviposition, crop rotation, resistance

Damage to first year (rotated) corn by western corn rootworm, *Diabrotica virgifera virgifera* LeConte, has been reported in Illinois (Levine and Gray 1996), Indiana (Sammons et al. 1997), Iowa (Rice and Tollefson 1999), Michigan (DiFonzo 1998) and Ohio (Onstad et al. 1999). Formerly, female *D. v. virgifera* beetles were thought to oviposit only in cornfields, and since *D. v. virgifera* larvae can survive only on the roots of corn and a few grasses, crop rotation was a highly effective management practice (Levine and Oloumi-Sadeghi 1991). However, during the late 1980's, rootworm injury to corn following soybeans was reported in isolated areas of east central Illinois (Levine and Oloumi-Sadeghi 1996, Levine and Gray 1996). By 1995, *D. v. virgifera* larval injury to corn following soybeans was observed throughout east central Illinois and northwestern Indiana (Levine and Gray 1996, Onstad et al. 1999).

Entomologists hypothesized that widespread adoption of corn-soybean rotation had selected a variant of *D. v. virgifera* that lays at least a portion of its eggs in soybean fields (Levine and Gray 1996, Sammons et al. 1997, Spencer et al. 1999). In a corn-soybean rotation, larvae from eggs oviposited and overwintering in soybean, emerge in a cornfield the following spring. These first-year fields are typically not treated with a soil insecticide and can experience extensive root injury and yield reduction from *D. v. virgifera* larval feeding. Alternatively, eggs laid in cornfields hatch the following year in a non-host field (i.e. soybean). Thus, where corn is grown in rotation with soybeans there is an advantage for female *D. v. virgifera* to lay eggs in soybeans. Accordingly, O'Neal et al. (1999) found greater numbers of adult *D. v. virgifera* in soybean versus cornfields in east central Illinois, including a greater percentage of females in soybean fields than in cornfields during August (O'Neal et al. 1999), when *D. v. virgifera* likely laid the majority of their eggs (Hein and Tollefson 1985). Interfield dispersal of *D. v. virgifera* is common, with first-year cornfields a site of female immigration (Godfrey and Turpin 1983). While corn phenology has been shown to influence northern corn rootworm (*Diabrotica barberi* Smith and Lawrence) dispersal (Naranjo and Sawyer 1988), and is thought also to affect movement of *D. v. virgifera* (Naranjo 1991), its role in *D. v. virgifera* movement into soybeans has not been investigated.

Both Sammons et al. (1997) and Spencer et al. (1999) observed suspected variant *D. v. virgifera* adults feeding on soybean leaves. Specifically, Sammons et al. (1997) reported that suspected variant beetles from Indiana fed more on soybean leaves in a no-choice assay than *D. v. virgifera* from Iowa and Nebraska. They found that beetles from Nebraska did not feed at all on soybean leaves. In Illinois, Spencer et al. (1999) also reported field observations *D. v. virgifera* feeding on soybeans. They suggested that although a preference for soybeans is not necessary to explain rootworm oviposition in soybeans, it may be possible to identify *D. v. virgifera* that have oviposited in soybean fields by presence of soybeans in their gut.

Recent reports of injury to rotated corn in southwestern Michigan (DiFonzo 1998) suggest that variant populations are present in Michigan as predicted by Onstad et al. (1999). Our initial objective was to determine if feeding assays could identify the presence of variant *D. v. virgifera* within regional populations. We first evaluated if *D. v. virgifera* adults from the regions tested by Sammons et al. (1997) varied in their consumption of soybean in no-choice assays. Results from these tests led us to study *D. v. virgifera* acceptance for soybean when in the presence of corn at different phenological stages. Finally, we investigated whether the influence of corn phenology on soybean feeding is unique for *D. v. virgifera* from putative variant populations. Based on the results, we suggest a model that can explain *D. v. virgifera* dispersal from corn and oviposition in soybeans.

Materials and Methods

General assay conditions. We measured the surface area of corn and soybean leaves fed on by *D. v. virgifera* beetles in no-choice and choice assays based on the no-choice assay of Sammons et al. (1997), in which 10 adult beetles were presented individual soybean leaves within a petri dish. In our preliminary studies, as few as 1 in 10 individuals fed on a soybean leaf during a 24 h period; moreover, females fed more than males. Feeding by *D. v. virgifera* adults often occurs in groups, on both corn and soybeans. Therefore, we decided to use 10 adult female beetles in a petri dish as the experimental unit to assure measurable feeding would occur, and to allow us to compare our results to that of Sammons et al. (1997). Plant material and *D. v. virgifera* source varied among experiments, but unless otherwise specified, the following conditions were kept constant.

All beetles were kept in wire screen (30 cm by 30 cm by 30 cm) cages in an insect rearing facility (16:8 L:D, 24° C) on the campus of Michigan State University. To insure that exposure to soybean leaves in the assays was not a novel experience, beetles were provided with a potted soybean plant (V6 to R3 stage), as well as a fresh corn ears, silks, and leaves. Water was provided continuously from dental wicks placed in water-filled flasks. Beetles were taken from cages, briefly chilled on ice for easier handling, and then separated by sex based on the shape of the last abdominal sternite (White 1977). Beetles and test foliage were placed in petri dishes (15.5 cm diameter and 1 cm high) on filter paper (Whatman No. 2) moistened with water. Unless otherwise specified, foliage used in feeding assays was from the 3rd fully expanded leaf from the apex of both corn and soybeans. Beetles were allowed to feed for 24 h in a growth chamber (16:8 L:D, 24° C). After 24 h, beetle mortality was recorded and the leaf area consumed was digitally measured using the public domain NIH Image software (Anon. 1999). Every beetle survived the 24 h assay; therefore leaf area consumed is reported as a mean per dish.

No-Choice Assay. In 1999, our objective was to differentiate variant from wild type *D. v. virgifera* using no-choice assays. We conducted no-choice assays with beetles from Illinois, Nebraska and Michigan. Based on the findings of Sammons et al. (1997), we hypothesized that *D. v. virgifera* from Illinois (variant) would feed on soybeans and those from Nebraska (wild type) would not. We further postulated that feeding by Michigan beetles might be an intermediate between Nebraska and Illinois, based on whether they were predominately of a variant or wild type population.

Insects Adult *D. v. virgifera* were collected from cornfields in Nebraska (Saunders County, 30 July, 1999; 2 mi from the collection site of Sammons et al. 1997), a region of corn production that has not reported rootworm larval injury to rotated corn. *Diabrotica virgifera virgifera* from Michigan were collected in a continuous cornfield at the Michigan State University Entomology Research Farm (East Lansing, MI), also a region where *D. v. virgifera* injury to rotated corn has not been reported. Beetles from Illinois were collected from a rotated cornfield in Champaign County in a region frequently reporting injury to rotated corn (O'Neal et al. 2001). Beetles from Nebraska were sent to Michigan in containers with corn ears, silks, water, and arrived within 48 h of collection. Beetles from Illinois and Michigan were collected 48 h before the arrival of those from Nebraska; Illinois beetles were transported by car in cages with corn ears, silks, and water.

Plants Soybeans used for all feeding assays (Asgrow Ag2201) were planted on 12 May at the Michigan State University Entomology Research Farm (East Lansing, MI) and maintained using conventional agronomic practices. Soybeans used in the no-choice assay were in the late R2 to early R4 stage (Sinclair and Backman 1989).

Experimental Design and Data Analysis Beetles were offered a single soybean leaf within an 8.5 cm diameter and 1.5 cm high petri dish. No-choice assays were performed with females from each state on three separate dates: 2, 3, and 9 August 1999. Each treatment was replicated three times on each of the 3 d. Results from the 3 d were combined and analyzed using analysis of variance (ANOVA) for a randomized complete block design, with assay date as the block, state of origin as the treatment and the interaction between the block and treatment included in the ANOVA. The amount of soybean leaf foliage consumed was compared across the three treatments (*D. v. virgifera* from Illinois, Nebraska and Michigan) using LSD mean separation (SAS 1996).

Corn phenology assay. To determine if the propensity for *D. v. virgifera* to feed on corn leaves changed as corn plants age and which phenological stages of corn elicit the greatest feeding by *D. v. virgifera*, we conducted a series of choice assays with corn leaves from four unique phenological stages (Ritchie et al. 1986). In this experiment, beetle source and age were held constant while corn age varied. We conducted these assays on three separate days with corn leaves from 4 separate plantings of corn. The three assay dates provided comparisons of corn in early to late vegetative stages, vegetative to reproductive stages, and early to late reproductive stages, respectively.

Insects *Diabrotica virgifera virgifera* were obtained from a laboratory colony maintained at the Northern Grain Insects Lab, Brookings, South Dakota. Emerging teneral adults were collected during a 48 h period then sent overnight to Michigan in containers with food (butternut squash and a fructose-glycerine diet) and water. Beetles were delivered on 21 March, 4 and 18 April, 2000. During each assay, beetles were approximately 14 d old based on adult emergence occurring the day of shipment.

Plants Corn used in the corn phenology assay (Pioneer 3573) was planted every 10 d in a greenhouse (16L : 8D) from 1 February to 2 March for a total of four separate plantings. Before each assay, corn phenology was quantified according to Ritchie et al. (1986). Leaves used in the assay were the numerical middle leaf determined from the total number of leaves with fully formed collars. Sections of leaf tissue (6 cm by 3 cm) cut from either side of the midrib were used.

Experimental Design and Data Analysis In each petri dish, beetles were offered an 18 cm^2 section of leaf from each of the four planting dates. Assays were conducted on three separate dates (4 April, 17 April and 2 May). On each date, five replications were conducted. Two separate ANOVA were used to determine first, if corn age affected the leaf area consumed and second, which phenological stage within an assay was fed on most. In the first analysis, data from the three assay dates were combined and analyzed as a randomized complete block design, blocked by assay date with planting date as the treatment factor. The interaction between assay date and planting date was included in the analysis. Assay dates and planting dates with significantly different amounts of leaf area consumed were identified using LSD mean separation (SAS 1996). In the second analysis, individual assay dates were analyzed as a completely randomized design to determine which phenology stages were consumed in the greatest amount. Phenology stages with significantly different amounts of leaf area consumed were identified using LSD mean separation.

Corn-soybean choice assays. Based on results of the corn phenology assay, we inferred that corn phenology may influence *D. v. virgifera* consumption of soybean leaves. We postulated that soybean leaf consumption would be greater in the presence of corn from a phenological stage not readily eaten by *D. v. virgifera* (late vegetative to reproductive stage) than in the presence of corn from a phenological stage more readily eaten (early vegetative stage). We further postulated all post-reproductive stages of corn (R1- R6) would have a similar effect on soybean consumption. We also tested *D. v. virgifera* feeding on soybean leaves in the presence of corn silks. Because *D. v. virgifera* feeding on corn silks is more commonly observed than feeding on leaves, we tested the hypothesis that soybean leaf feeding occurs only in presence of corn foliage and not in the presence of corn silks.

Insects *Diabrotica virgifera virgifera* used in the corn-soybean choice assays came from a continuous cornfield on the Michigan State University Entomology Research Farm (East Lansing, MI). Adult beetles were collected by hand from corn plants the day prior to assay.

Plants Corn used in the corn-soybean choice assays was planted on 29 April and 15 May, 2000. As with the corn phenology assay, a 6 cm by 3 cm section of corn leaf tissue cut from either side of the midrib was used in the corn-soybean choice assays. Soybean was planted on 16 April. A whole soybean leaflet was taken from the third fully expanded trifoliate, below the apex of the plant. We were interested in how corn phenology influences soybean consumption, and not in comparing corn and soybean leaf consumption by *D. v. virgifera*. Therefore, we did not attempt to offer equal mass or surface area of each leaf type.

Experimental Design and Data Analysis Petri dishes contained a section of corn leaf from one of the two planting dates along with a full soybean leaf. Assays were conducted on three separate dates (21 July, 29 July and 3 August), to compare feeding on corn at differing phenological stages. On the first assay date, leaves came from corn in either early or late vegetative stages. On the second assay date, leaves came from corn in either the reproductive or mid-vegetative stages. The final assay date, leaves came from corn in early or late reproductive stages. On 3 August, corn silks were tested in a separate choice test. Corn silks were taken from plants in the R1 or R2 stage, representing fresh versus older silks (browning) respectively. Silks from plants in the R1 stage were selected if that plant was shedding pollen, and silks from plants in the R2 stage were selected if pollen shedding had ceased. Silks were cut from the corn-ear tip and weighed. The weight of corn silk provided was equal to the weight of the soybean leaf included in the petri dish. On 21 and 29 July, 10 replications of each choice assay were conducted, while on 3 August only seven replications were conducted for each of the four choice assays.

For each assay date, data were analyzed separately as a completely randomized design with type of plant material (corn or soybean) eaten as the treatment factor. ANOVA was used to test the effect of corn phenology on corn consumption, and corn phenology and organ type (silk vs. leaf) on soybean consumption (SAS 1996). A student *t*-test compared the amount of soybean consumption when the silks presented came from R1 versus R2 corn plants.

Regional corn-soybean choice assays. Finally, we tested whether corn phenology (early vegetative or reproductive stage) affected corn and soybean consumption of *D. v. virgifera* from different regions. Beetles from Illinois (putative variant), Nebraska (putative wild type), and Michigan were analyzed using the corn-soybean choice assay.

Insects During the summer of 1999, we tested beetles from Nebraska and Illinois that were collected for the no-choice assay. In 2000, we again used beetles from the same locations in Illinois and Nebraska, as well as Michigan beetles from our corn-soybean choice assay. *Diabrotica. virgifere virgifera* from Illinois and Michigan were collected 48 h before the arrival of those from Nebraska (5 August, 2000), with Illinois beetles transported by car containers with corn ears, silks, and water.

Plants In 1999, corn used in the feeding assays was planted at the Michigan State University Entomology Research Farm (East Lansing, MI) on two dates, 15 May and 23 June. When used in the assay, corn planted on 15 May was in the R2 stage, and corn planted on 23 June was in the V5 to V6 stage (Ritchie et al. 1986). Soybean leaves were taken from the same field as those used in the 1999 no-choice assay. In 2000, soybean planted for the corn-soybean choice assays was used. In 2000, corn used in the regional choice assays came from the 29 April planting used in the corn-soybean choice assay and additional planting made on 30 May, to insure a source of vegetative stage corn.

Experimental Design and Data Analysis On 8 August 2000, we conducted choice assays with beetles from Michigan, Illinois and Nebraska. An intact soybean leaf was offered along with a 6 cm by 3 cm section of corn leaf, with 10 replications for each state. The statistical analysis followed that of the corn-soybean choice assay, with separate analyses conducted for *D. v. virgifera* from each state.

Results

No-choice assay. Beetles from each location consumed measurable amounts of soybean foliage (Fig. 1). Overall, there was a significant effect on the amount of soybean leaf area consumed due to the *D. v. virgifera* source ($F = 6.24$, df = 2, 18; $P = 0.01$) as well as an interaction between assay date and *D. v. virgifera* source ($F = 3.56$, df = 4, 18; $P = 0.03$). Female *D. v. virgifera* from Michigan consumed more leaf area than beetles from Nebraska. There was no significant difference in the amount of soybean leaf area eaten between beetles from Illinois and Nebraska.

Corn phenology assay. Based on the first analysis of the corn phenology assays, we observed a significant effect of assay date on the total amount of corn consumed during each assay ($F = 11.4$, df = 2, 48; $P < 0.01$). The total amount of corn consumed decreased from the first assay date to the third assay date (Fig. 2), suggesting an overall effect of corn age on *D. v. virgifera* feeding. There was also a significant effect of planting date on *D. v. virgifera* feeding ($F = 7.73$, df = 3, 48; $P < 0.01$). More leaf area was consumed from corn planted on the latest date (Table 1, 2 May) than on any of the other three planting dates. In addition, there was a significant interaction between assay date and planting date ($F = 9.59$, df = 6, 48; $P < 0.01$).

In our second analysis of the corn phenology assay (Fig. 2), there was a significant effect of phenology on the amount of corn leaf area eaten within each of the three assay dates (First assay, $F = 16.89$, df = 3, 16; $P < 0.01$: Second assay, $F = 16.82$, df = 3, 16; $P < 0.01$: Third assay, $F = 3.65$, df = 3, 16; $P = 0.04$). During the first two assays, the youngest corn (V6-V7) was consumed in significantly greater amounts than the older corn stages (Fig. 2). In the third assay, the third planting date was consumed the most, however, the overall feeding was greatly reduced during this assay.

Corn-soybean choice assay. There was significantly more feeding on the younger of the two corn leaves (Fig. 3), when corn phenology varied from early to late vegetative stages (21 July, $F = 4.86$, df =1,18; $P = 0.04$), and when the corn phenology varied from vegetative to reproductive stages (29 July, $F = 4.53$, df = 1, 18; $P = 0.05$). There was no difference in amount of *D. v. virgifera* feeding when both corn leaves were taken from plants in reproductive stages (3 August, $F = 2.08$, df = 1,12; $P = 0.18$).

Corn phenology also influenced soybean consumption, with more soybean area eaten when in the presence of the older corn leaves (Fig. 3). *D. v. virgifera* feeding on soybean was greater when in the presence of the late versus early vegetative stage corn leaves (21 July, $F = 5.26$, df = 1, 18; $P = 0.03$). This trend was present in the latter two assays, but was not significant when leaves were from late-vegetative and reproductive stage corn (29 July, $F = 1.71$, df = 1, 18; $P = 0.21$) and only marginally so when both leaves came from reproductive stage corn (3 August, $F = 4.36$, df = 1, 12; $P = 0.059$).

Diabrotica virgifera virgifera feeding on soybean was significantly reduced in the presence of corn silks (Fig. 4, $F = 61.56$, df = 1, 27; $P < 0.01$). However, feeding on soybean occurred in 12 of 14 replications where silks were present. Although the extent of feeding on silks was not measured, there was significant evidence of feeding on silks from both the R1 and R2 stage corn. Silks were still available in each treatment at the end of the 24 h assay. A significantly greater amount of soybean was consumed (t-test, $t = 3.7$, df = 7, $P < 0.01$) in the presence of silks from corn in the R2 stage (no pollen shed) than from corn in the R1 stage (pollen shedding).

Regional choice assay. Again in 2000 (Fig 5), feeding on corn ($F = 76.47$, df = 1, 18; $P < 0.01$) and soybean ($F = 48.10$, df = 1, 18; $P < 0.01$) leaves by Illinois beetles was influenced by corn phenology. Illinois beetles ate more younger than older corn and fed more on soybeans in the presence of older than younger corn. Michigan beetles consumption of corn ($F = 76.23$, df = 1, 18; $P < 0.01$) and soybean ($F = 48.10$, df = 1, 18; $P < 0.01$) was also influence by corn phenology. Finally, though Nebraska beetles consumption of corn leaves was influenced by corn phenology ($F = 83.58$, df = 1, 18; $P < 0.01$), the affect on the consumption of soybean was not significant ($F = 3.30$, df = 1, 18; $P = 0.09$).

Discussion

No-choice assays. Unlike Sammons et al. (1997), in our tests *D. v. virgifera* from Nebraska readily fed on soybean leaves and beetles from Illinois (putative variant *D. v.*

virgifera) did not consume a statistically greater amount of soybean foliage than beetles from Nebraska (wild type *D. v. virgifera*). Rather, we consistently observed that *D. v. virgifera* consumed soybean foliage regardless of source population. Feeding on soybean occurred in both no-choice and choice assays, even when corn silks, considered highly attractive to *D. v. virgifera*, were offered (Fig. 4). Feeding on soybeans by *D. v. virgifera* is also not unique to the confines of a petri dish. We observed beetles from all three populations feeding on soybeans in holding cages. We also observed Michigan populations in the field feeding on soybean plants. *Diabrotica virgifera virgifera* in Illinois (Spencer et al. 1999), and Nebraska (L. Mienke, personal communication) have also been observed to feed on soybean plants in the field. These observations suggest that adult feeding on soybeans is not unique to *D. v. virgifera* taken from areas where oviposition in soybeans is common.

Corn phenology assays. From the corn phenology assay we observed that corn age affected *D. v. virgifera* feeding on leaves. Across the three assay dates, the last date had the lowest level of feeding. On this date, three of the four leaves offered were from corn in a reproductive stage. During each assay date, among the different stages of corn offered to *D. v. virgifera* (Fig. 2), we observed that leaves from corn in the early to mid-vegetative (V6- V7) stage were fed on more than those from late vegetative (V10) to reproductive stages (R1 - R3). Overall, our results support and extend those of Sifuentes and Painter (1964) who found that delaying the plantings of similar lines of corn by a month decreased leaf feeding by corn rootworms. Our results suggest that *D. v. virgifera* feeding on corn leaves decreases as the plant enters the late vegetative to reproductive stage. The interaction between assay date and planting date can be accounted for by the secc

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second planting of corn, which elicited the greatest amount of feeding by *D. v. virgifera* during the third assay. Why the third planting date (21 February) was consumed more during the third assay is not clear. In the third assay, the variability in amount of leaf area fed on was reduced across all but the second planting of corn. Given the overall lower amount of feeding during the third assay, the corn phenology assay would be sensitive to variability in beetle behavior within replications. Accordingly, leaf surface eaten in two of the five replications of the second planting date in the third assay were twice that of the average for the entire second planting date.

Corn-soybean choice assays. After establishing *D. v. virgifera* prefer feeding on corn leaves from early vegetative stage than those from corn in a reproductive stage, we examined if this finding might influence *D. v. virgifera* feeding on soybeans. As predicted, we observed *D. v. virgifera* consumption of soybean was greater in the presence of phenologically older versus younger corn. We refer to this as a phenological affect.

It is well known that *D. v. virgifera* prefer to feed on floral parts (Krysan 1986) of corn when they are present. We investigated whether *D. v. virgifera* would feed on soybeans in the presence of corn silks, and if the phenological effect observed with corn leaves could be produced with silks. Though *D. v. virgifera* fed less on soybean leaves when silks instead of corn leaves were present, feeding on soybean leaves still occurred (Fig. 4). In addition, a phenological effect was present when the silks came from older plants (i.e. more soybean leaf area was eaten).

Regional choice-assays. We again compared *D. v. virgifera* from Illinois and Nebraska in choice tests with old and young corn. Though conducted almost a week apart, the

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2000 regional choice assay (Fig. 5) differed from the 3 August, 2000 corn-soybean choice assay (Fig. 3), in that the corn offered was selected to produce a phenological effect. In 2000, beetles from all three states fed more on younger corn (Fig. 5). Consistent with our previous three assays (Fig. 3), Michigan beetles consumption of soybean was influenced by corn phenology. Illinois and Nebraska beetles feeding on soybeans was decidedly lower than that of Michigan beetles, though both tended to feed more on soybean in the presence of older corn leaves. Therefore, we conclude that the influence of corn phenology on soybean feeding is not unique to beetles from Michigan. However, only Illinois and Michigan beetles fed significantly more on soybean when the alternative was older corn. Nebraska beetles followed the same trend, though the relationship was not statistically significant.

A model for *D. v. virgifera* oviposition in soybeans. Sammons et al. (1997) hypothesized that corn residue accumulating on the soil surface due to reduced tillage might attract *D. v. virgifera* into soybean fields. However, olfactometer results were not consistent with this hypothesis, and they concluded that corn residue was not responsible for attracting *D. v. virgifera* into soybean (Sammons et al. 1997). They suggested that injury to rotated corn was due to a variant *D. v. virgifera* that has a preference for soybean environments but did not suggest a mechanism for such a preference. Spencer et al. (1999) investigated if suspected variant *D. v. virgifera* adults preferred soybeans to corn in a flight chamber. Overall, a greater percentage of females were collected on corn than soybean. This occurred even though corn tassels and silks, both sources of volatile attractants (Metcalf 1986), were removed from corn used in their flight chamber studies.

Spencer et al. (1999) concluded that suspected variant *D. v. virgifera* do not have an attraction for soybean plants.

Regarding *D. v. virgifera* ovipositional habitats, all the cues necessary to induce *D. v. virgifera* oviposition in soybeans are apparently present. Field and laboratory studies have consistently demonstrated that damp particulate substrate is sufficient to induce *D. v. virgifera* oviposition. Gustin (1979) observed more than a 4-fold increase in oviposition in corn plots with high (24.9 percent moisture) versus low (16.2 percent moisture) soil moisture. Kirk et al. (1969) demonstrated in the laboratory that damp covered soil with a large particle size (12.5 - 25.0 mm) is sufficient to induce females to oviposit. Of these factors, moisture had the greatest influence (Kirk et al. 1969). Kirk et al. (1969) concluded that South Dakota *D. v. virgifera* had no inherent attraction to corn as an oviposition site. More recently, Siegfried and Mullin (1990) observed that caged *D. v. virgifera* from Pennsylvania would oviposit in the absence of corn. They also concluded that oviposition does not appear to be regulated by cues on or near a host plant and suggest that adult feeding and oviposition are separate behaviors. Thus it appears that given appropriate soil moisture, *D. v. virgifera* oviposition is as likely in a soybean field as a cornfield.

Given the potential for indiscriminate ovipositional behavior of *D. v. virgifera* and a lack of attraction to soybeans, understanding adult dispersal from cornfields becomes key in explaining injury to rotated corn. *Diabrotica virgifera virgifera*, especially females with mature eggs, have been observed to move out of the cornfields from which they emerge and enter rotated cornfields (Godfrey and Turpin 1983). Also, later-planted (phenologically young) corn can function as a trap crop drawing in large numbers of adult corn rootworm late in the season as surrounding fields mature (Naranjo and Sawyer 1988, Hill and Mayo 1974). These observations suggest *D. v. virgifera* commonly move in and between cornfields so as to find plants of a phenological stage acceptable for feeding. Our studies demonstrate that *D. v. virgifera* feed little on late vegetative or reproductive stage corn foliage and, in its presence, choose to feed on soybean foliage. While silks briefly offer a preferred food source, a preference for silks does not preclude soybean feeding. Thus, where corn is planted over a short period of time in the spring and is mostly of a phenologically similar stage, *D. v. virgifera* are presented with the following scenario. As corn matures and becomes a less acceptable food, *D. v. virgifera* will likely disperse. In areas of strict corn-soybean rotation, they will either encounter corn in a stage as unattractive as the field from which they left or, alternatively, encounter a soybean field.

The larger numbers of *D. v. virgifera* found in soybean than cornfields, as observed by O'Neal et al. (1999), may thus be due primarily to dispersal from cornfields with a phenology that is unattractive as a feeding site. The response of *D. v. virgifera* to corn phenology may be particularly strong in areas of extensive corn and soybean rotation, especially where corn is planted with a high degree of synchrony. A prediction from the corn phenology model is that oviposition in soybeans is not unique to *D. v. virgifera* from any particular geographic area. Rather, any corn-rotated landscape with highly synchronized planting resulting in phenologically similar corn, should encourage *D. v. virgifera* dispersal into the non-host (in this case, soybeans). An additional prediction of the corn phenology model, is that successful oviposition in a corn-soybean rotated landscape is possible without an attraction to or a preference to feed on soybeans.

This may help explain the occurrence of damage to first year corn in northeastern Iowa (Rice and Tollefson 1999), an area well outside the predicted range of the variant *D. v. virgifera* (Onstad et al. 1999).

While the corn phenology model does not require a genetic component, there is a pattern of spread in damage to first year corn originating in east central Illinois that is consistent with the diffusion of a genetic change in regional *D. v. virgifera* populations (Onstad et al. 1999). We refer to this as the genetic variation model. *Diabrotica virgifera virgifera* that oviposit in soybean will have a selective advantage as their offspring have a higher probability of survival, and thus, selection could produce a variant. However, no genetic differences between such beetles and the wild type have yet been detected. Sammons et al. (1997) suggested that a variant would have an increased affinity for soybeans. Spencer et al. (1999) refuted this, suggesting that survival in a corn-soybean rotated landscape does not require an attraction to soybeans, but rather a relaxed fidelity to corn. By combining this refined genetic variation model from Spencer et al. (1999) with the corn phenology model, we suggest that a decreased fidelity to corn, mediated by corn phenology, is likely the primary behavior selected for in a corn-soybean rotated landscape. We suggest that in areas of extensive corn-soybean rotation, especially with highly synchronous planting (as is common in east central Illinois) a corn phenology effect may have increased *D. v. virgifera* dispersal and consequent incidental oviposition in soybeans. An increased acceptance of soybean, for either feeding or oviposition, could be a secondary adaptation, if present at all.

Conclusion

We conclude that adult *D. v. virgifera* feeding on soybeans *per se* is not a reliable predictor of potential oviposition in soybeans, as this behavior was not unique to *D. v. virgifera* from areas where corn-soybean rotation fails to protect corn from *D. v. virgifera* larval injury. Rather, we found that *D. v. virgifera* feeding on corn leaves is highly influenced by the phenological stage of the plant, and that reduced acceptability of corn influences *D. v. virgifera* feeding on soybeans. We suggest that in regions of extensive corn-soybean rotation, corn phenology is responsible for the greater occurrence of *D. v. virgifera* in soybeans, and postulate that oviposition occurs coincidentally while adults visit soybean fields. Given that the affect of corn phenology on soybean feeding was not unique to *D. v. virgifera* from areas where corn-soybean rotation has failed to protect corn, the predictions of a corn phenology model for *D. v. virgifera* dispersal and oviposition in soybean fields does not require the assumption of a genetic variant. However, the advanced development of corn when adult *D. v. virgifera* emerge in a corn-soybean landscape would increase wild type *D. v. virgifera* oviposition in soybeans as less time is spent in the aging corn. Over time, these conditions would select for a variant *D. v. virgifera* with a decreased fidelity to corn. To identify a variant that is resistant to a corn-soybean rotation, we suggest examining physiological (i.e. time to adult emergence) or behavioral differences (i.e. sensitivity to corn phenology) that would produce a greater response to corn phenology in a variant than the wild type.

Table 1. Mean (± SEM) corn leaf area eaten by *D. v. virgifera* in corn phenology assays, organized by corn planting date

Planting date	corn phenologies tested[b]	cm^2 (± SEM)[c]
1 February, 2000	VT, R1, R3	0.05 (± 0.03) b
11 February, 2000	V10, VT, R2	0.56 (± 0.16) b
21 February, 2000	V8, V9, R1	0.58 (± 0.17) b
2 March, 2000	V6, V7, V10	2.41 (± 0.55) a

[a]Corn planted in a greenhouse on the campus of Michigan State University.

[b] phenology ratings from Ritchie et al. (1986) were determined at each of three assay dates (4 April, 17 April and 2 May, 2000).

[c] Values followed by a different letter are significantly different (LSD, $P = 0.05$).

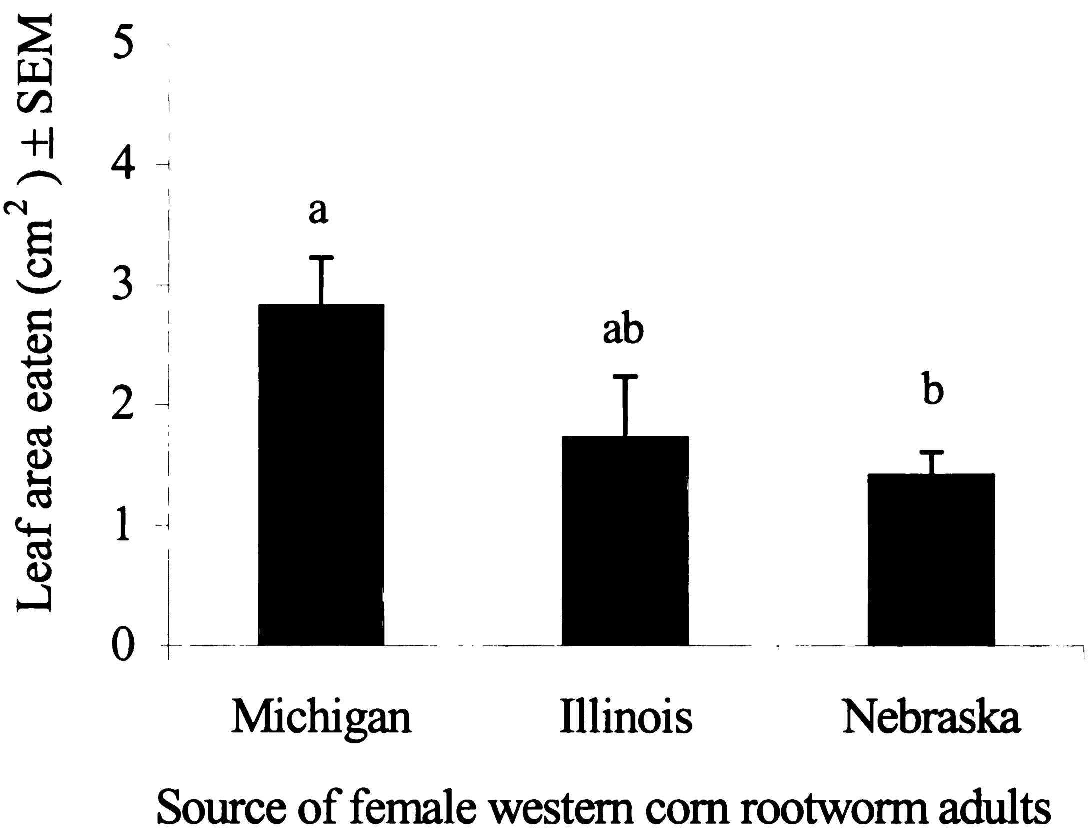

Fig. 1. Mean (± SEM) soybean leaf area eaten by 10 female western corn rootworms from Michigan, Illinois and Nebraska in a 24 h no-choice assay. Means labeled with the same letter are not significantly different (LSD, P = 0.01).

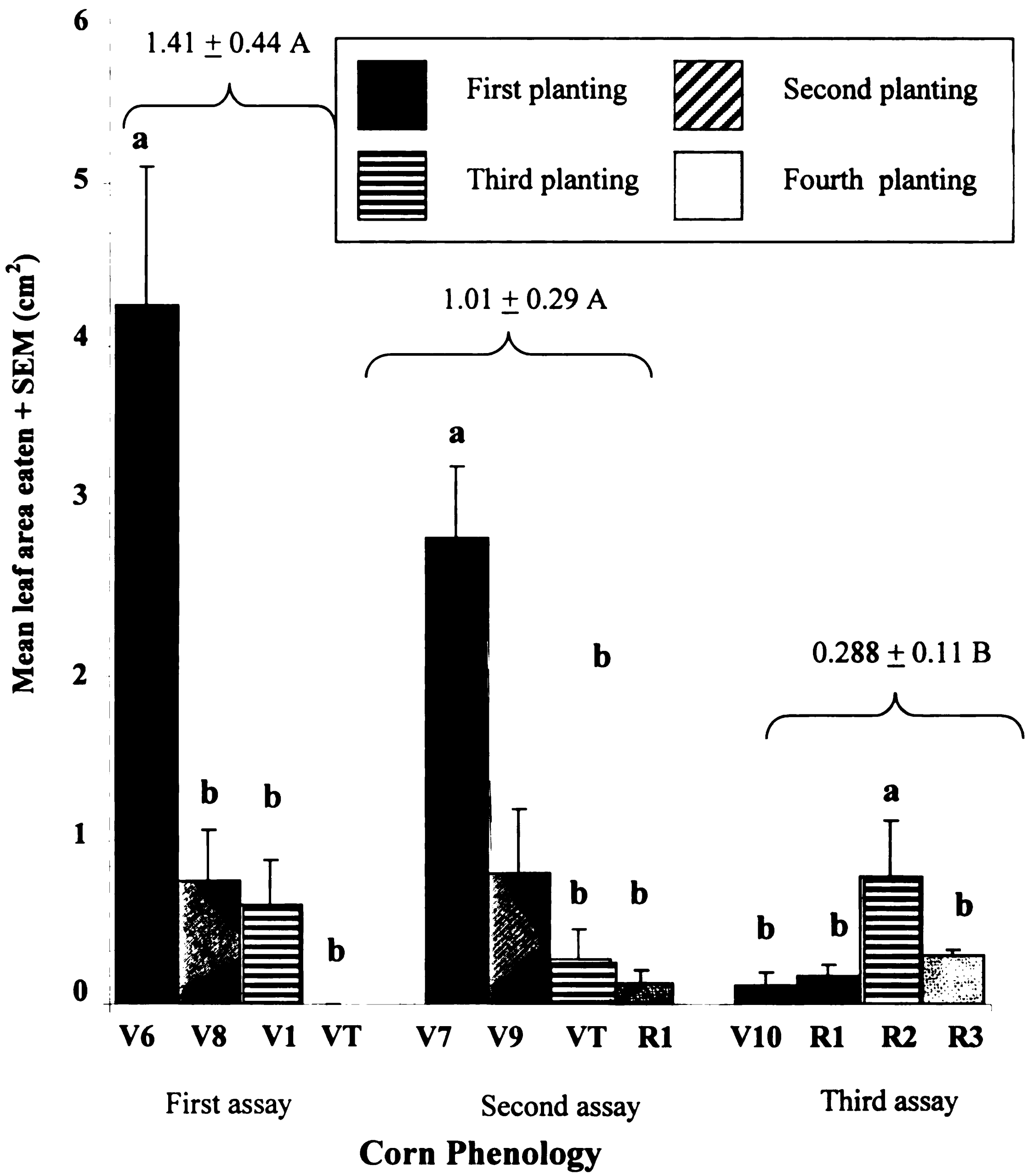

Fig. 2. Mean (± SEM) corn leaf area eaten by 10 adult female western corn rootworms in a choice assay of four leaves from four separate planting dates. The first planting date is indicated with a clear bar, second with horizontal strips, third with diagonal strips and the fourth date with a gray bar. The phenology for each planting is designated on the y-axis. The combined mean (± SEM) for the leaf area eaten during the assay date is indicated above the brace. Means for the assay dates labeled with the same capital letter are not significantly different (LSD, $P < 0.05$) and means for the planting dates labeled with the same lower case letter are not significantly different (LSD, $P < 0.05$).

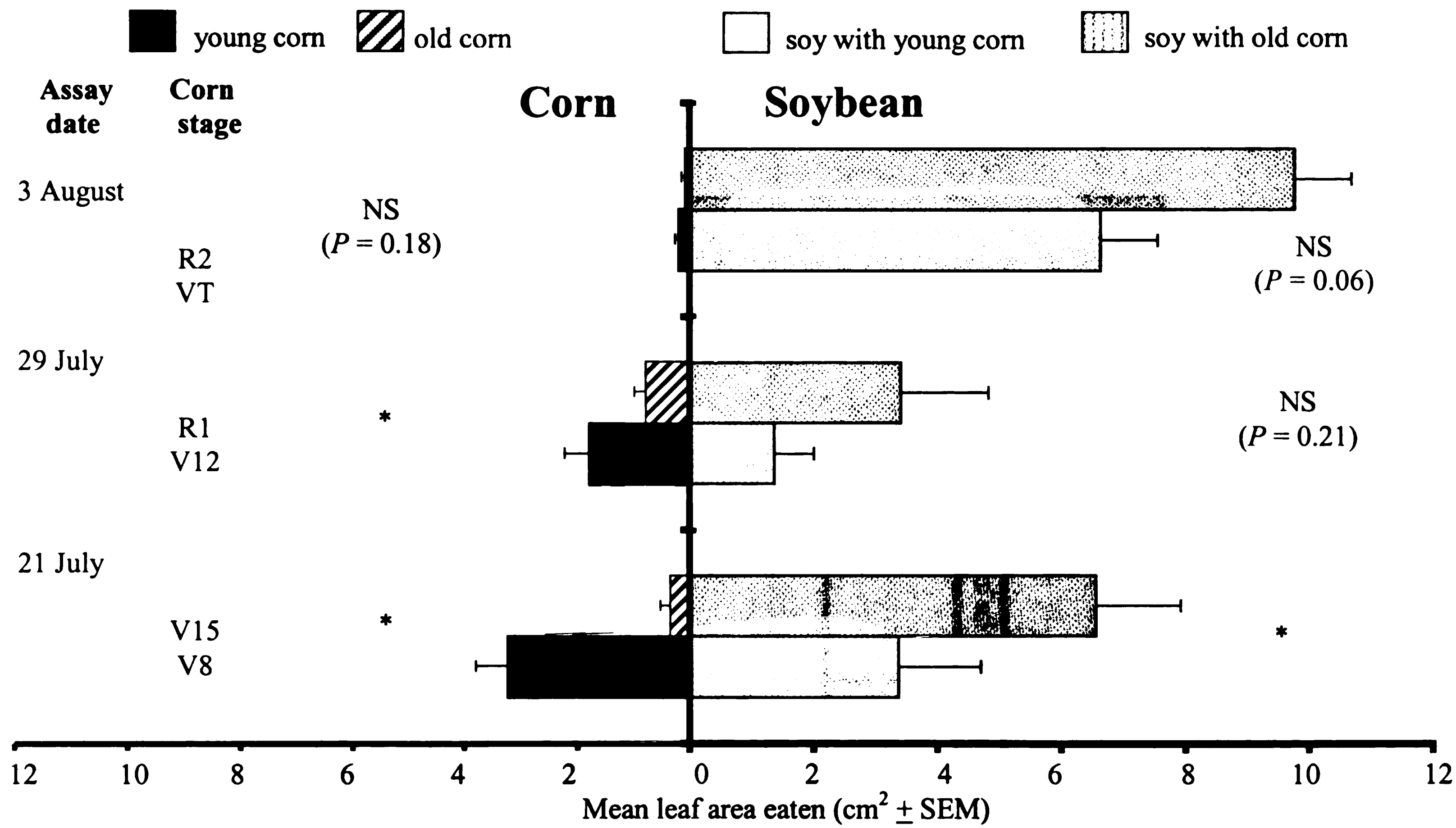

Fig. 3. Corn and soybean leaf areas eaten by *D. v. virgifera* in a choice assay. Corn leaves came from younger corn indicated by black horizontal bar and older corn indicated by vertical strips. All soybean leaves came from the same planting date, with gray bars indicating soybean offered with younger corn and gray vertical strip soybean offered with older corn. Timing of the three assays produced a comparison of early vs. late vegetative corn (21 July), late vegetative vs. reproductive stage corn (29 July), and early vs. late reproductive stage corn (3 August). Asterisks indicate if corn phenology affected corn or soybean consumption (*, $P < 0.05$; ** $P < 0.01$).

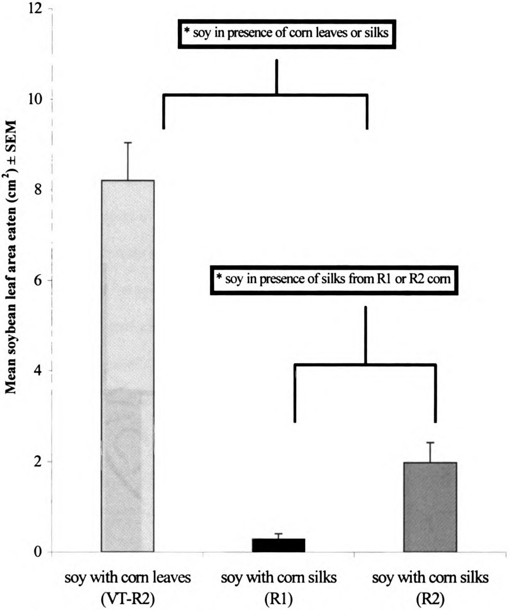

Fig. 4. Mean (± SEM) soybean leaf areas eaten by 10 adult female western corn rootworms when offered with either corn leaves or silks. Silks from corn in the R2 stage were taken from plants that were no longer producing pollen, and silks from R1 corn were producing pollen.

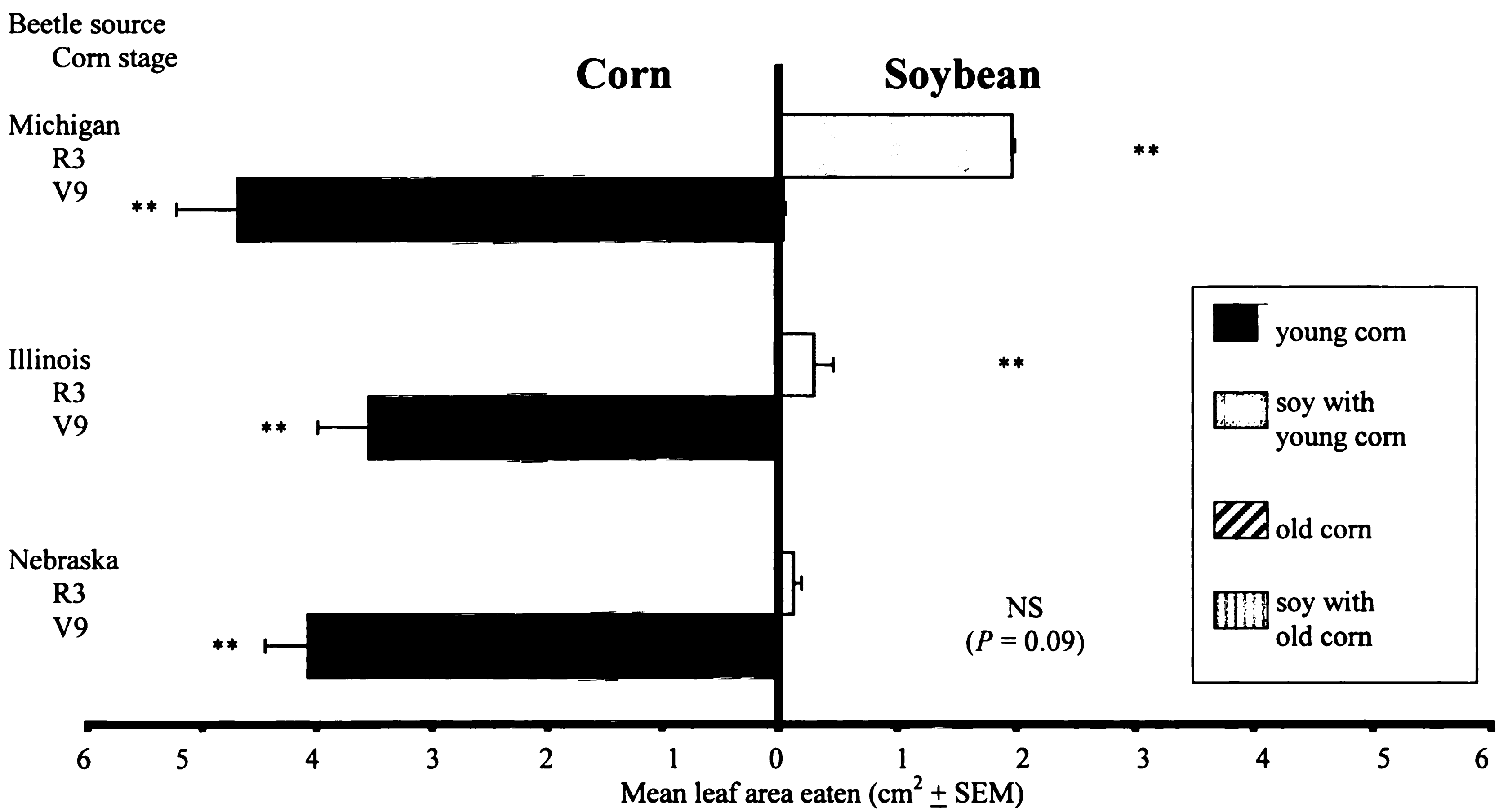

Fig. 5. Corn and soybean leaf areas eaten by female *D. v. virgifera* from three populations. Leaves were offered in a choice assay, with soybean leaves from one planting date and either reproductive stage (R3) or vegetative stage (V9) corn leaves. Black horizontal bar indicates leaves from vegetative stage corn and vertical strips indicate the reproductive stage. Gray bar indicates soybean offered with vegetative stage corn, gray vertical strip soybean offered with reproductive stage corn. Asterisks indicate if corn phenology affected corn or soybean consumption (*, $P < 0.05$, **, $P < 0.01$).

CHAPTER 5:

DISPERSAL RESPONSE OF *DIABROTICA VIRGIFERA VIRGIFERA* (COLEOPTERA: CHRYSOMELIDAE) TO CORN OF VARYING PHENOLOGY

ABSTRACT: One hypothesis to explain the mechanism of rotation-resistance by adult western corn rootworms (*Diabrotica virgifera virgifera* LeConte) is that corn phenology influences adult dispersal. We report the development and use of a dispersal assay to characterize the response of adult *D. v. virgifera* to corn structures of varying phenology. After verifying that the assay measured adult response to phenology, we tested if this response differed between wild-type and putative rotation-resistant *D. v. virgifera*. Beetles from a region of Nebraska where injury to rotated corn has not been reported, served as our wildtype, and those from a region of Illinois were injury to rotated corn is common were considered rotation-resistant. We compared the retention of *D. v. virgifera* on a sham leaf to corn structures of varying phonological stages (young or old leaf section, young or old tassel), for a total of five treatments. There was a significant treatment effect ($F = 7.6$, df = 4, 24; $P = 0.001$), with the sham leaf retaining the fewest adult female *D. v. virgifera* in the dispersal bioassay. Both putative rotation-resistant and wild type beetles were less likely to disperse from young tassels (shedding pollen) than old tassels (not shedding pollen). We did not observe an effect of beetle origin ($F = 1.05$, df = 1, 48; $P = 0.32$), or an interaction of beetle origin ($F = 0.12$, df = 1, 48; $P = 0.74$) with any treatment. We observed a greater response, in terms of the distribution of *D. v. virgifera* adults in the dispersal assay, to old than young tassels. Distribution of beetles in

a cage with old tassels differed significantly from a 1:1 ratio (X^2= 3.94, df = 1, P = 0.04), but did not when young tassels were present (X^2= 0.9, df =1, P = 0.34). Although corn is an adequate host for *D. v. virgifera*, even its most attractive phenological stage is not sufficient to prevent adult beetle dispersal.

KEY WORDS: crop rotation, resistance, behavioral bioassay

The failure of crop rotation to protect corn in parts of Illinois and Indiana from larval rootworm injury has been attributed to the oviposition of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in soybean. The current explanation (Levine and Gray 1996, O'Neal et al. 1999, Spencer et al. 1999) is that female *D. v. virgifera* move out of the cornfields from which they emerge, move into soybean fields and oviposit throughout these fields (O'Neal et al. 2002). Oviposition in soybeans can be accompanied with feeding on soybean foliage. Although feeding on soybeans was first attributed to *D. v. virgifera* that have a proclivity for oviposition in soybeans (Sammons et al. 1997). Feeding on soybean foliage is not unique to *D. v. virgifera* from areas where crop rotation failure is common (O'Neal et al. 2002), and as such is not predictive of a tendency to oviposit in soybeans.

O'Neal et al. (2002) observed that *D. v. virgifera* feeding on soybean increased in the presence of post-reproductive versus early-vegetative corn. Leaves and silks of post-reproductive stage corn also increased feeding on soybean leaves. The influence of corn phenology on the consumption of soybean foliage led O'Neal et al. (2002) to suggest that it is this response that is responsible for the apparent expansion of *D. v. virgifera* oviposition sites to include soybean fields.

Diabrotica virgifera virgifera responds to corn phenology in several ways. Greater numbers of adult *D. v. virgifera*, especially females are found in late planted cornfields in younger phenological stage than surrounding cornfields (Darnell et al. 2000). Individual *D. v. virgifera* have a greater tendency to fly towards corn of a younger phenological stage (Naranjo 1991). Volatile components of corn have been identified as attractants (Metcalf 1986, Hammack 1997), and are produced by plants during pollination when silks and tassels are present.

Furthermore, oviposition in the absence of a host plant is not uncommon and has been recorded in the lab and field for *D. v. virgifera* (Kirk et al. 1969, Gustin 1979, Branson and Krysan 1981, Siegfried and Mullin 1990). Oviposition is not considered to be dependent upon a cue from a larval host-plant (Siegfried and Mullin 1990). A landscape that is planted early and synchronously to large acres of corn and soybean would produce a population of *D. v. virgifera* likely to leave corn and enter soybeans with the potential to oviposit therein. Thus, the critical step in determining if an individual oviposits in soybeans may be how it responds to the phenology of the corn in which it emerged.

The apparent spread of injury to rotated corn (Onstad et al. 1999) suggests that a unique variety of *D. v. virgifera* resistant to crop rotation originated within east central Illinois. A single gene locus model is sufficient to explain the apparent development of this resistance (Onstad et al. 2001). This model does not suggest a behavioral mechanism, although it does require a high degree of a corn-soybean rotation in a landscape for a selective advantage to be realized by the resistant individuals. If *D. v. virgifera* respond to advanced corn phenology by dispersing into soybeans and thus ovipositing there, such an advantage would exist. Two potential mechanisms could produce such a resistant variety; a behavioral variant with a response to corn phenology that is greater than the wild type, or a variant that emerges later in the growing season and encounters older corn than the wild type. It is the former mechanism that we will explore in these studies.

Here we report the development and use of a dispersal assay to characterize the response of adult *D. v. virgifera* to corn structures of varying phenology. After verifying that the assay did measure adult response to plant phenology, it was employed to test three hypotheses. We first tested if crop stage or plant structure influenced dispersal and if this response differed between wild type and putative rotation-resistant *D. v. virgifera*. Finally, we compared the degree of response of *D. v. virgifera* to corn tassels of differing phenologies.

Materials and Methods

Insects In the spring of 2001, we tested the dispersal response of *D. v. virgifera* obtained from a laboratory colony maintained at the Northern Grain Insects Lab, Brookings, South Dakota. Emerging teneral adults were collected during a 48 h period then sent overnight to Michigan in containers with food (butternut squash and a fructose-glycerine diet) and water. Beetles were delivered on 11 April, 2001. During each assay, beetles were approximately 14 d old based on adult emergence occurring the day of shipment.

During the summer of 2001 and 2002, we compared the dispersal of *D. v. virgifera* collected from Nebraska and Illinois. Adults were collected from cornfields in Nebraska (Saunders County, 23 July, 2001; 2 km from the collection site of Sammons et al. 1997), a region of corn production that has not reported larval rootworm injury to rotated corn. Beetles were sent 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 in Champaign County in a region where injury to rotated corn is common (O'Neal et al. 2001), and transported by car in cages with corn ears, silks and water, 48 h before the arrival of those from Nebraska.

All beetles were kept in wire screen (30 cm by 30 cm by 30 cm) cages in an insect rearing facility (16:8 L:D, 24° C) on the campus of Michigan State University. Water was provided continuously from dental wicks placed in water-filled flasks. For the no-choice dispersal assay, beetles were given leaves and tassels from both vegetative and reproductive stage (defined below as young and old) corn. Females were placed into separate cages 24 h before being placed in the dispersal assay. Beetles were taken from cages, briefly chilled on ice for easier handling, and then separated by sex based on the shape of the last abdominal sternite (White 1977).

A sub-sample of beetles from each assay was saved and preserved as voucher specimens in the A. J. Cook Arthropod Research Collection at Michigan State University.

Plants Corn and soybean was grown at the Michigan State University Entomology Research Farm (East Lansing, MI) and maintained using conventional agronomic practices. We planted corn on four dates (23 April, 7, 20 and 28 May, 2001) to insure a supply of vegetative and reproductive stage corn. Soybean was planted on 5 May 2001.

No-choice dispersal assay conditions. The dispersal assay cage was constructed with aluminum wire screen (18 by 16 mesh, 0.011 mm gauge) and a cardboard cylinder bottom (Fig. 1). The cage has an inner and an outer chamber. The inner chamber (30 cm high by 14.5 cm diameter) walls are wire screen with clear plastic ends. The top of the inner chamber has a 4.5 cm diameter hole at its center. The floor and 10 cm of the outer chambers' wall is cardboard, with the walls covered in fluon coated aluminum foil. The remainder of the 50 cm high by 24 cm diameter outer chamber is constructed of wire screen. An 18 cm high cone-shaped lid that ends in a 2 cm diameter opening also constructed from wire screen rests on top of the outer chamber. The top of the lid opens into an 11 cm by 8 cm diameter clear plastic container with a wire screen top.

We measured adult *D. v. virgifera* dispersal response to corn structures of differing phenologies in the inner chamber of the dispersal assay cage. Corn leaves or tassels were placed in a 50 ml Erlenmeyer flask and the top sealed with parafilm. Two 45 cm long sections of corn leaf or one 45 cm long tassel, measured from the tip, were placed within the flask. Leaves were taken from corn in early vegetative stage (V6-8 stage, based on Ritchie et al. 1986 scale, referred to as 'young leaves') or late vegetative stage (V14-VT, based on Ritchie et al. 1986 scale, referred to as 'old leaves'). Tassels cut from early reproductive stage corn (VT, referred to as 'young tassels') were used only while they were producing pollen. Conversely, tassels from late reproductive stage corn (R2, referred to as 'old tassels') were used only if they were no longer producing pollen.

Ten adult beetles were placed in the inner chamber with the upper hole blocked with a clear plastic lid. After 60 minutes, the lid was removed and the outer chamber sealed. Unless otherwise stated, the number of adults in the inner chamber was counted after 30 minutes, 3 hours and 24 hours. Every assay started at noon so that assayed beetles would experience a full scotophase. All assays were run at room temperature (24° C) and a 16:8 L:D photoperiod.

Experimental design and data analysis.

Assay validation We first tested if the retention of adult females in the inner chamber was due to the treatment presented or to some inherent property of the dispersal cage. We compared the retention of *D. v. virgifera* on a sham leaf to that of corn structures of varying phenologies (young or old leaf section, a young or old tassel) within our dispersal assay. This sham was constructed from a 6 cm by 40 cm long section of cardboard approximately the same height as the leaf treatments, exposing 105 cm^2 surface. In each cage, 10 lab colony beetles were offered one of five treatments and each treatment was replicated five times. After 24 hours, the number of beetles remaining in the inner chamber was counted. We used a completely randomized design to evaluate the effect of the five treatments on rootworm retention, and accounted for treatment effects using ANOVA with least significant differences to compare retention across treatments (SAS 1998). The amount of leaf area eaten was also measured for the two real leaf treatments using the method developed by O'Neal et al. (2002). The difference in amount of leaf area consumed between the two leaf treatments was compare using Student t tests with Satterthwaite correction (SAS 1996).

Regional test During the summer of 2002, our objective was to differentiate rotation-resistant from wild type *D. v. virgifera* using the dispersal assay. Based on the corn phenology model (O'Neal et al. 2002), we hypothesized that dispersal of western corn rootworms from Illinois (rotation-resistant) from corn of an advanced phenology would be greater than that of those from Nebraska (wild type). First, we compared these populations separately, to determine their response to the four treatments (young and old corn leaves and tassels). Later, both populations were compared at the same time to determine if the degree of response to the corn phenologies differed both across treatments and within a given treatment.

In the first trial, ten female adults were assayed in dispersal cages with four treatments: old corn leaves, young corn leaves, old tassels and young tassels. Each plant organ treatment was replicated five times. Illinois beetles were tested 48 h after collection in Champaign County (8 August) on 10, 12 and 14 August. Nebraska beetles were also tested 48 h after collection in Saunders County (14 August) on 16, 18, and 20 August. The effect of plant organ on beetle retention was analyzed using analysis of variance (ANOVA) for a randomized complete block design, with assay date as the block, and the interaction between time (number in inner chamber after 30 min, 3 hr, 24 hr) and treatment included in the analysis. To determine when a treatment effect occurred, we used the time by plant organ interaction as an error term to slice the treatment effect at the three time levels (30 min, 3 hr, 24 hr).

In the second trial, we tested if Illinois and Nebraska beetles responded differently to old and young tassels by simultaneously testing them during a single 24 hr period. Each treatment was replicated seven times, to increase assay robustness and reveal potential differences in beetle response to corn phenology. We compared the response of beetles from each population in two separate analyses. The first analyses accounted for the effect of plant organ on dispersal using ANOVA for a 3 by 3 factorial design, with population of origin, time (number in inner chamber after 30 min, 3 hr, 24 hr) and plant organ as treatment factors. The second analyses compared how the distribution within a given treatment varied for our putative variant and wild type populations. A mean observed ratio of beetles in the inner to the outer chamber was calculated for each state and plant organ combination and compared to a 1:1 ratio with a Chi-square goodness of fit test (X^2, Sokal and Rohlf 1995).

Results

Assay validation. There was a significant treatment effect on the dispersal of adult female *D. v. virgifera* across the five different treatments ($F = 7.6$, df = 4,24; $P = 0.001$). Significantly fewer females were found on the sham leaf compared to the plant structures (Fig. 2). In two of the five replicates, no beetles remained on the sham leaf. The sham was the only treatment for which this was true. Young tassels (i.e. those shedding pollen) retained the most females, with significantly fewer beetles on older tassels that were no longer shedding pollen. Although there was no difference in the dispersal from young and old corn leaves, we did measure a significantly greater amount of leaf area consumed ($t = 2.78$, df = 4; $P = 0.05$) by females on the young leaf (7.94 ± 2.62, mean $cm^2 \pm$ SEM) than the old leaf treatment (1.29 ± 0.54, mean $cm^2 \pm$ SEM).

Regional test. In general, the least amount of dispersal occurred when both our putative variant and wild type beetles were given young tassels. There was a significant effect of plant structure on the retention of Nebraska beetles (Table 1). More Nebraska beetles were found in the inner chambers with young tassels after 3 h and 24 h (Table 1 and Fig. 3a). Although we did not see a significant effect of plant structure on the retention of Illinois beetles, we did see a significant time by plant interaction (Table 1). The slice of the time by plant effect on time, revealed a significant effect at 24 h. This effect is observed in the figure 3b, where significantly more Illinois beetles were found on young tassels.

Since we did not see a difference in the number of Illinois or Nebraska beetles in inner chambers containing young or old corn leaves (Fig. 3), we did not include leaf treatments in our second trial. When we compared Illinois and Nebraska beetles simultaneously, we observed a significant effect of time and plant organ on beetle retention (Table 2, Fig. 4). We did not observe an effect of beetle origin (Table 2), or a significant interaction of beetle origin with any factor. Illinois and Nebraska beetle's response to young and old tassels was identical (Fig. 4). Within cages, the distribution of beetles from both Illinois and Nebraska followed a similar pattern. The distribution of beetles in a cage with old tassels differed significantly from a 1:1 ratio indicating their lack of preference for older tassels, but did differ significantly when young tassels were present (Table 3).

Discussion

To confirm that beetles response was due to the differences in treatments and not affected by the cage design, we compared corn leaves and tassels to a sham leaf. Beetles were more likely to leave the inner chamber when the sham-leaf was presented than any corn organ. On average, 1.6 beetles per cage were present within the inner cage after 24 hr, but two of the five replicates had no beetles within the inner chamber. This was not the case for any other treatment. Since the sham leaf alone did not retain a significant number of beetles, this suggests that the cage design is appropriate for measuring the dispersal response of *D. v. virgifera* to the corn structure placed within it.

O'Neal et al. (2002) demonstrated that *D. v. virgifera* preferred to feed on corn leaves from early vegetative stage plants ('young') over leaves from reproductive stage corn ('old'). Although we observed a similar preference for young corn leaves in the amount eaten, we did not observe a difference in retention between the young and old leaf treatment in our dispersal assay. This was true for *D. v. virgifera* from a laboratory colony in South Dakota, as well as those from Illinois and Nebraska.

Young tassels were the most consistent in retaining the most beetles, for all three populations tested. However, old tassels produced the greatest deviation from an even distribution of beetles between the inner and outer chambers (Table 3). This indicates that even in the presence of the most attractive stage of corn, dispersal activity continues to occur. Beetle dispersal is significantly higher when old tassels are present. This suggests that older corn has a strong influence on beetle dispersal and even the most attractive plant stage does not retain all individuals

We predicted that rotation-resistant would show an increased sensitivity to corn phenology and thus disperse more readily from older corn stages than wild type beetles. This was not the case. Thus we are unable to separate putative wild type and rotation-resistant *D. v. virgifera* populations based on a phenology-mediated dispersal response. However, the response of both populations was consistent with our proposed mechanism for the oviposition of *D. v. virgifera* within soybean fields. Given our results, we would expect a high likelihood of dispersal by *D. v. virgifera* within an agroecosystem with large acreage of post-reproductive stage corn. O'Neal et al. (2002) demonstrated that the presence of post-reproductive stage corn produced greater feeding on soybean leaves. What influence previous multiple experiences with an unattractive host plant have on the likelihood and degree of dispersal and oviposition of *D. v. virgifera* into soybeans is not known.

We further conclude that we were unable to separate our putative wild type and variant *D. v. virgifera* populations based on a phenology-mediated dispersal response.

Although this is not conclusive, it does leave open the hypothesis that a rotation-resistant variety may not exist. As mentioned in the introduction, the oviposition behavior of *D. v. virgifera* is not highly restricted, with oviposition produced in the lab in soil around host and non-host alike. Therefore, factors that influence the distribution of adults would be crucial in determining the distribution of eggs. A high degree of reliance on a corn-soybean rotation with a corn crop that is planted early and in a highly synchronous manner could produce a shift in *D. v. virgifera* oviposition sites out of corn and into soybean.

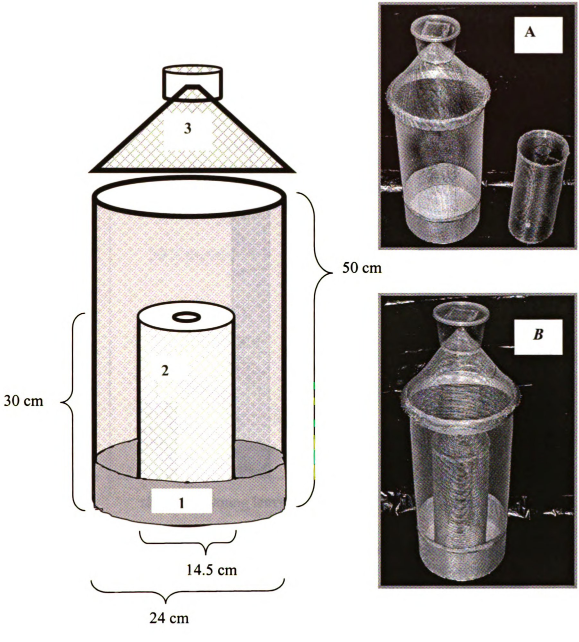

Fig. 1. Diagram of the dispersal assay. 1, outer chamber with fluon coated aluminum foil covering the 10 cm of the inner wall. 2, Outer chamber with a 4.5 cm hole in its clear plastic top. 3, cone-shaped removable top of outer chamber. Inner and outer chambers are shown separate (A) and together (B).

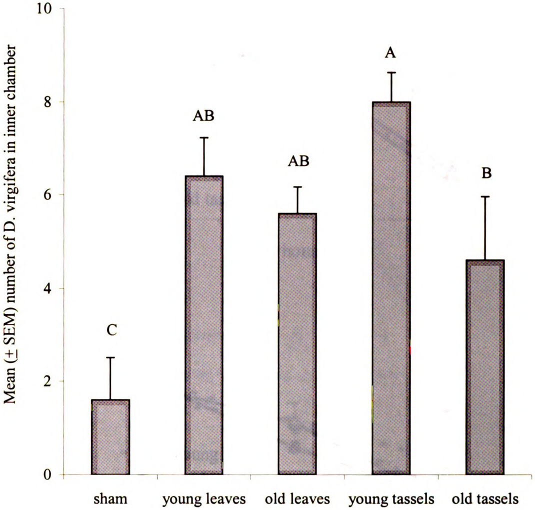

Fig. 2. Dispersal response after 24 hr by *D. v. virgifera* from laboratory colony to young corn leaves (mid-vegetative stage), old corn leaves (reproductive stage), young tassels (shedding pollen), old tassels (no pollen), and a sham leaf. Bars with same letter are not significantly different according (LSD, $P \leq 0.05$).

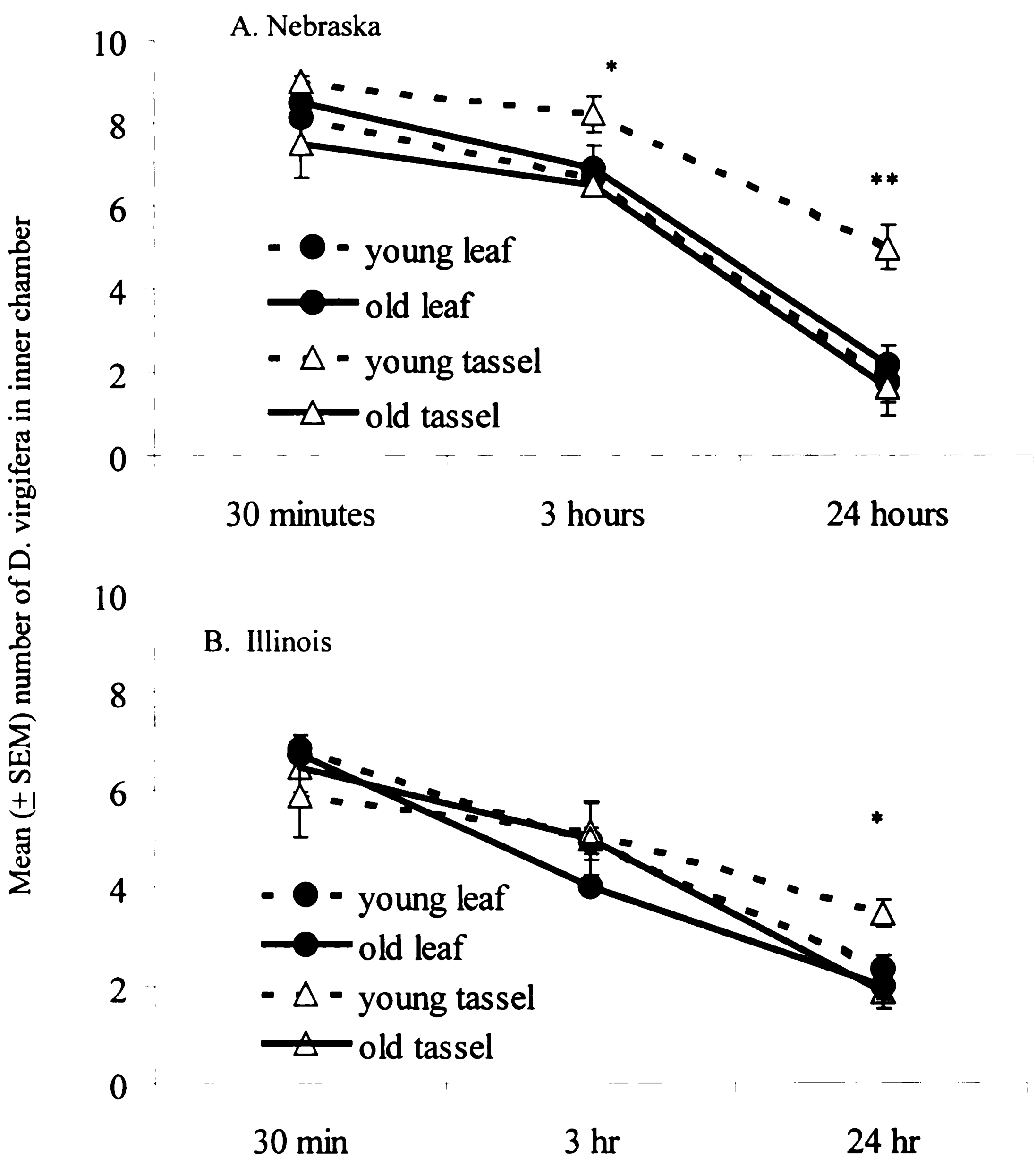

Fig. 3. Dispersal response after 30 min, 3 hr and 24 hr by *D. v. virgifera* from Nebraska (A) and Illinois (B) to young corn leaves (mid-vegetative stage), old corn leaves (reproductive stage), young tassels (shedding pollen), and old tassels (no pollen). Asterix indicate a significant treatment effect within a sampling period (ANOVA, *, $P \leq 0.05$; **, $P \leq 0.01$).

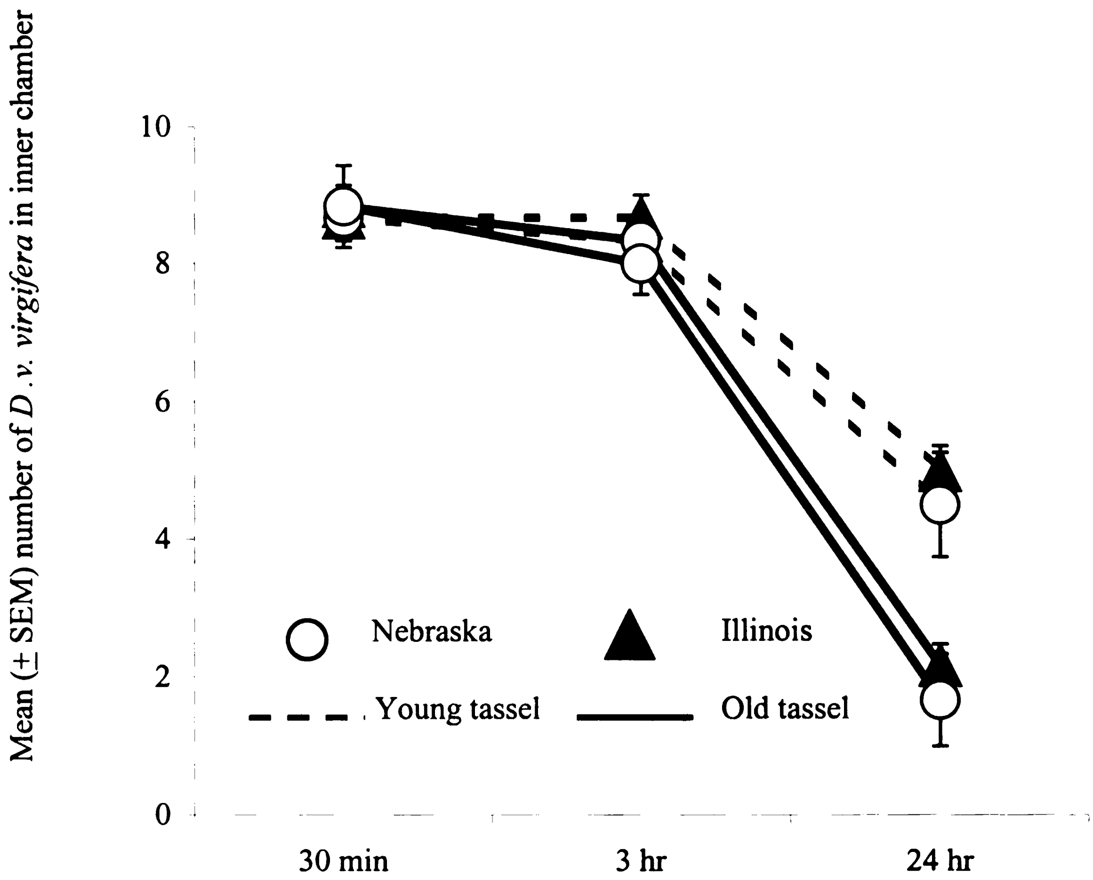

Fig. 4. Dispersal response after 30 min, 3 hr and 24 hr by *D. v. virgifera* from Nebraska and Illinois to young tassels (shedding pollen) and old tassels (no pollen).

Table 1. Effect of corn structures of varying phenology part on the dispersal of two populations of *D. v. virgifera* at 3 time periods

	Nebraska			Illinois		
Source	df	*F*	*P*	df	*F*	*P*
Block	2	27.71	<0.0001	2	7.64	0.001
Time	2	65.52	<0.0001	2	100.83	<0.0001
Plant structure	3	11.66	<0.0001	3	1.24	0.30
Time * Plant	6	1.20	0.31	6	2.39	0.03
Slice of Time *Plant effect by time						
	Nebraska			Illinois		
Time	df	*F*	*P*	df	*F*	*P*
30 minutes	3	1.80	0.15	3	3.20	0.32
3 hours	3	2.72	0.05	3	1.19	0.19
24 hours	3	9.54	<0.0001	3	1.62	0.03

Table 2. Comparison of Illinois and Nebraska *D. v. virgifera* dispersal response to corn structures of varying phenology at 3 time periods

Source	df	*F*	*P*
Origin	1	1.05	0.32
Time	2	141.38	<0.0001
Plant structure	1	17.66	0.0001
Time * Origin	2	0.35	0.71
Origin * Plant structure	1	0.12	0.74
Time * Plant structure	2	7.37	0.002
Time * Origin * Plant structure	2	0.27	0.76

Table 3. Distribution of female *D. v. virgifera* in a two-chamber dispersal assay when presented corn tassels of varying phenologies

State	Tassel age[a]	Observed ratio (inner : outer chamber)	X^2	*P*
Illinois	old	1 : 4.5	3.94	0.04
	young	1 : 1.8	0.9	0.34
Nebraska	old	1 : 4.9	4.36	0.04
	young	1: 1	4.34×10^{-14}	1

[a] Young tassels were taken from plants producing pollen, old tassels were taken from plants two weeks past pollen shed.

CHAPTER 6:
CONCLUSION:
A LANDSCAPE EXPLANATION FOR THE FAILURE OF CROP ROTATION

ABSTRACT: Early explanations for the failure of crop rotation to manage *D. v. virgifera* proposed a genetically distinct 'rotation-resistant' population. However, the geographic spread of rotation-resistance has not matched early predictions. To date, efforts to separate rotation-resistant from wild type populations have been unsuccessful. Several lines of evidence suggest that sufficient behavioral plasticity of wild type populations that emerge within a landscape of mature corn and limited crop diversity may lead to rootworm injury to rotated corn. Corn phenology has been shown to influence *D. v. virgifera* dispersal from corn and acceptance of soybeans, thus an alternative explanation that does not require existence of a rotation-resistant population may be appropriate. *Diabrotica virgifera virgifera* immigration and subsequent oviposition in soybeans may be due to a corn crop in an advanced phenological stage. Using USDA data, we describe general trends in the timing of corn planting in several key Corn Belt states over a thirty-year period. A consistent trend was observed across several states for earlier planting. In the region where injury to rotated corn is common, this early-planted corn crop is in a landscape of limited diversity, composed almost entirely of corn and soybeans. Based on these trends and data from previous studies, I propose an alternative explanation that landscape structure can account for the apparent development of a rotation-resistant *D. v. virgifera*, without invoking a genetically distinct population. The landscape described above would be expected to increase wild type *D. v. virgifera* dispersal to soybeans, promoting the failure of crop-rotation. Thus, by adjusting

landscape structure to reduce emigration from cornfields, the effectiveness of crop rotation could be increased.

Since 1986, western corn rootworm (*Diabriotica virgifera virgifera* LeConte) has been reported to cause injury to corn (*Zea mays* L.) planted after soybeans (*Glycine max* L.) (Levine and Oloumi-Sadeghi 1996) throughout East-Central Illinois and Northwestern Indiana (Onstad et al. 1999). Rotation is practiced to a high degree in this region of the Corn Belt, and, prior to 1995 was successful at preventing rootworm injury to rotated corn. *Diabriotica v. virgifera* have survived and caused injury to corn in this corn-soybean rotated landscape through an expansion of their oviposition range to include soybean fields (Levine et al. 2002). Before the 1990's, oviposition by the univoltine *D. v. virgifera* was considered to occur primarily in cornfields (Levine and Oloumi-Sadeghi 1991), thus this expansion has been attributed to the emergence of a population distinct from the wild type. *Diabrotica v. virgifera* capable of injuring rotated corn have been described as 'rotation-resistant,' implying a genetic change like that associated with insecticide resistance.

Initial predictions overestimated the geographic spread of rotation-resistance (Onstad et al. in review). Furthermore, efforts to separate rotation-resistant from the wild type populations have been unsuccessful (Spencer et al. 1999, Hibbard et al. 2002, O'Neal et al. 2002). With evidence that corn phenology influences *D. v. virgifera* dispersal from corn and acceptance of soybeans as an adult feeding site (O'Neal et al. 2002), an alternative explanation that does not require existence of a genetically distinct rotation-resistant population may be appropriate. Herein, I suggest that the synchronous, earlier planting of corn combined with limited crop diversity may favor oviposition of *D. v. virgifera* in soybean. Under these conditions, the potential for rotation-resistance is an emergent property that all *D. v. virgifera* populations may display. While selection may occur resulting in a genetically distinct population, such a population is not a pre-requisite to explain the current occurrence of injury to rotated corn.

The 'Rotation-Resistance' Hypothesis:

Onstad et al. (1999) described the initial incidence and geographic occurrence of injury to rotated corn by *D. v. virgifera*. Results from Onstad et al. (1999) spatial modeling supported the hypothesis that a population of *D. v. virgifera* capable of circumventing a corn-soybean rotation originated in Ford County, Illinois in 1986 (Levine and Oloumi-Sadeghi 1996). Onstad et al. (1999) could account for the occurrence of large numbers of western corn rootworms in soybean fields in a four state region by 1997 by assuming that a rotation-resistant population had spread from this center of origin along weather patterns typical for East-Central Illinois. Onstad et al. (2001) used a population-genetic model based on a single-gene locus for rotation-resistance to explain the development of this population in East-Central Illinois. The almost singular use of a corn-soybean rotation in this area was considered the selection pressure that produced the rotation-resistance variety of *D. v. virgifera*.

Although modeling efforts by Onstad et al. (2001) did not explicitly describe the behavioral mechanism employed by rotation-resistant beetles to circumvent rotation, they did suggest that rotation-resistance based on greater adult acceptance for soybean was unlikely. Since *D. v. virgifera* from areas where rotation failures are common, are unable to survive as larvae on soybean roots, fitness costs are too high to maintain a population with a greater preference for soybeans in the adult stage (Onstad et al. 2001).

To date, efforts to identify a rotation-resistant phenotype have not been successful. Initial reports identified a putative rotation-resistant population with a greater visitation and acceptance for soybeans (Sammons et al. 1997). However, subsequent studies failed to confirm that adult *D. v. virgifera* from regions where injury to rotated corn is common select soybean over corn (Spencer et al. 1999). O'Neal et al. (2002) demonstrated that adult consumption of soybean leaves was not unique to beetles collected in such a region. Furthermore, electroantennogram response to soybean volatiles did not differ between putative rotation-resistant and wild type populations (Hibbard et al. 2002).

Given the lack of evidence for an attraction to soybeans, investigations into the mechanism for rotation-resistance have focused on dispersal from corn as the driving factor. Spencer et al. (1999) first proposed this explanation after failing to observe an attraction to soybeans by adult *D. v. virgifera*. Such a mechanism relies on a decreased fidelity to corn by adult *D. v. virgifera.* Because oviposition by *D. v. virgifera* is indiscriminate with regard to the presence of host/non-host cues, movement out of corn in a landscape that is predominately rotated corn and soybean would allow those females that oviposit in soybeans to realize a selective advantage.

Diabrotica v. virgifera **Dispersal**

Emigration by adult *D. v. virgifera* from cornfields in which they emerged is common. Adults, especially females with mature eggs, have been observed to move from fields where they emerge and enter rotated cornfields that do not have an existing population (Godfrey and Turpin 1983). Like most *Diabrotica* species, *D. v. virgifera* prefer to feed on floral parts (Krysan 1986) and floral volatiles have been shown to be highly attractive (Metcalf 1986). It is also well known that later-planted (phenologically young) corn, which 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 1988). Greater numbers of adult *D. v. virgifera*, especially females are found in late-planted cornfields than surrounding cornfields (Darnell et al. 2000), and individuals have a greater tendency to fly towards corn of a younger phenological stage (Naranjo 1991). Volatile components of corn have been identified as attractants (Metcalf 1986, Hammack 1997), and 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.

Oviposition by *D. v. virgifera*

Field and laboratory studies have consistently demonstrated that a damp, particulate substrate is sufficient to induce *D. v. virgifera* oviposition (Kirk et al. 1969, Gustin 1979, Branson and Krysan 1981, Siegfried and Mullin 1990). Kirk et al. (1969) concluded that *D. v. virgifera* had no inherent attraction to corn as an oviposition site. More recently, Siegfried and Mullin (1990) suggested that oviposition is not regulated by cues on or near the host plant, and that adult feeding and oviposition represent separate behaviors. Consequently, it appears that gravid *D. v. virgifera* females are likely to lay eggs where they happen to be feeding or resting, rather than searching for specific oviposition sites.

Corn Phenology and *D. v. virgifera* Acceptance of Soybean

O'Neal et al. (2002) demonstrated in choice tests that *D. v. virgifera* acceptance of soybeans as a food source increases as corn matures. The surface area of soybean foliage eaten by adult *D. v. virgifera* was greater in the presence of corn foliage in late-vegetative to early reproductive stage than corn foliage in early-vegetative stages. Feeding on soybean alone was not unique to beetles from a putative rotation-resistant population. Rather, *D. v. virgifera* from both Nebraska and Michigan, areas where injury to rotated corn has not been reported, fed as much if not more than adults from east-central Illinois. Corn phenology affected feeding on soybean for both wild type and putative rotation-resistant populations of *D. v. virgifera*. Because adult feeding on soybean increases with increasing age of corn, O'Neal et al. (2002) suggested that this is the source of the reduced fidelity that Spencer et al. (1999) suggested leads to oviposition in soybean.

Rotation Failure due to Corn Phenology

If the wild type *D. v. virgifera* populations' fidelity to corn declines as the plant ages, than this response could be selected to improve fitness in a landscape of rotated corn and soybeans. O'Neal et al. (in prep; see also Chapter 5), hypothesized that rotation-resistant adults may have an increased sensitivity to corn phenology. This would manifest itself in an increased dispersal from older stages of corn in comparison to wild type adults. This was not the case, however, as putative wild type and rotation-resistant *D. v. virgifera* populations have similar dispersal response to corn phenology. However, the response of both populations was consistent with the proposed mechanism for the oviposition of *D. v. virgifera* within soybean fields, i.e. both populations were less likely to disperse from young tassels (shedding pollen) than old tassels (not shedding pollen) (O'Neal et al. in prep).

Although reduced fidelity to corn due to advancing corn phenology cannot identify rotation-resistant *D. v. virgifera*, the behavior is a possible mechanism leading to ovipostion in soybeans. This explanation was outlined by O'Neal et al. (2002), who suggested that it is the dispersal response to corn phenology that is the primary behavior that drives oviposition in soybeans. Oviposition in soybeans is likely to occur within a landscape that is predominately corn and soybean fields when adult *D. v. virgifera* emerge in cornfields in a phenological state that increases emigration and acceptance of soybeans. Synchronous early planting of large acreages would produce corn in such a phenological stage. If the corn encountered by emerging adults were unattractive (i.e. post anthesis), then the likelihood of beetle dispersal would be high.

Synchrony of Rootworm and Corn Phenology

If corn phenology is the initial driver for *D. v. virgifera* dispersal from corn, then the timing of adult emergence and corn anthesis may be critical for predicting subsequent oviposition in soybeans. The importance of silks and tassels as adult feeding sites and their influence on rootworm reproductive success has been demonstrated both in laboratory (Elliott et al. 1990) and field experiments (Elliott et al. 1991). Female longevity and egg production increased when adults were feed corn ears and tassels at anthesis as opposed to those pre or post-anthesis (Elliott et al. 1990). Within artificially infested field plots, Elliott et al. (1991) observed the majority of rootworm eggs were laid within the period of corn anthesis. Elliott et al. (1991) conclude that temporal synchrony of corn anthesis with adult *D. v. virgifera* emergence is critical for understanding beetle dispersal and ovipostion.

Given the importance of corn phenology on *D. v. virgifera* survival and oviposition, one might expect rootworm development to be tightly linked to the growth of corn. Both *D. v. virgifera* and corn growth is dependent upon heat-unit accumulations, and can be predicted by degree-day models. However, from the review below, it is not clear that there is a direct synchrony between rootworm and corn phenology.

Models exist for predicting *D. v. virgifera* egg hatch (Schaafsma et al. 1991), adult emergence (Bergman and Turpin 1986, Elliott et al. 1990, Davis et al. 1996, Nowatzki et al. 2002) and population dynamics (Hein and Tollefson 1987, Elliott and Hein 1991). In general, laboratory (Wilde 1971, Levine et al. 1992) and field studies (Bergman and Turpin 1984, Hein et al. 1988, Fisher et al. 1990) indicate that larval development is affected by, but is not strictly a function of soil temperature (Davis et al. 1996). Synchronization of rootworm development with corn phenology is subject to host availability, yet there is no evidence that rootworm egg-hatch is dependent upon the presence of corn roots (Bergman and Turpin 1984). A delay in corn planting results in an extended adult emergence and lower populations of rootworms (Musick 1980, Bergman and Turpin 1984) attributed to larval mortality before corn roots are available.

When planting dates are synchronous, *D. v. virgifera* phenology does not respond to hybrid-based differences in corn development (Stavkisky and Davis 1997). Fewer first instar larvae and adults were found on earlier maturing hybrids than later maturing hybrids. This pattern may appear counter-intuitive since earlier maturing hybrids produced a greater number of root nodes in fewer growing degree-days. The slower root growth of later maturing hybrids produced a more substantial root system resulting in greater survival of later hatching eggs. As a result, adults emerged for a longer period of time from later maturing hybrids.

Stavkisky and Davis (1997) did not observe a correlation between adult emergence and corn anthesis. Although adult rootworms emerged sooner from faster maturing hybrids, it occurred after these hybrids had tasseled. Only beetles emerging from the longest maturing hybrid did so during corn anthesis. In general, hybrid maturity class did not affect the timing of peak adult emergence.

The timing of corn planting and anthesis is critical for larval survival and adult reproductive success respectively. However, it appears that rootworm development is not triggered by the presence of any given developmental stage of corn. Several factors can disrupt this relationship including corn planting date (Musick et al. 1980, Bergman and Turpin 1984), corn maturity class (Stavkisky and Davis 1997), and regional difference in degree-day accumulation (Davis et al. 1996). Predicting the likelihood of oviposition by *D. v. virgifera* in soybeans based on degree-day models of corn and beetle phenology may not be possible without incorporating these factors. Moreover, how these factors influence corn development at a regional level may be critical in estimating the risk of rotated corn to rootworm injury.

Agroecosystem Structure and Diabrotica v. virgifera Dispersal

Regional variation in corn production and landscape structure may intensify the influence of corn phenology on the dispersal of adult *D. v. virgifera* to soybeans. Although corn is produced throughout the North Central Region of the United States, injury to rotated corn is limited to East-Central Illinois and Northwest Indiana (Onstad et al. 1999 and in review). Below, I describe how the temporal and spatial heterogeneity of the agroecosystem in this region may increase the utilization of soybeans as an oviposition site by *D. v. virgifera.*

Recently, Onstad et al. (in review) have suggested that crop diversity may be a factor in slowing the apparent spread of putative rotation-resistant populations. Initial model predictions (Onstad et al. 1999) suggest a higher rate of spread when compared to actual observations for 1997 to 2001. New models that reduced *D. v. virgifera* dispersal in proportion to the amount of extra vegetation (farmland not planted to corn or soybean) within a county provided a better match to the 1997-2001 observations (Onstad et al. in review). In 1997, over 90% of the land in production in east-central Illinois was rotated corn and soybean, and less than 10% continuous corn (Onstad et al. 2001, from USDA census data). In contrast, counties that make up the southern border of Michigan, average only 50% rotated corn with little or no continuous corn. As of 2001, injury to rotated corn has been limited to the Southwestern corner of Michigan in Berrien County. However, with the incorporation of crop diversity these models still overestimate the geographic range where actual damage to rotated corn has occurred by approximately 40% (Onstad et al. in review).

Historical and regional trends in the production of corn are consistent with a landscape explanation for rootworm injury to rotated corn. Growers in the Midwest portion of the Corn Belt organize their planting schedule so that corn is planted at the earliest possible date based on soil moisture and temperature conditions. In the past, the range of planting dates for corn has aimed to produce plants that reach "knee high by the fourth of July." Those familiar with corn production are aware that this is no longer a useful axiom, with many states producing crops that are well past the mid-vegetative stage by early July. For example, an average mid-season commercial variety of corn planted in mid-May in Lafayette, Indiana is expected to reach anthesis by July (Neild and Newman 1990). Analysis of USDA crop progress data (Anon 2002) suggests that, for statewide averages, corn is planted earlier in 2000 than 1970 (Table 1). Although only Nebraska had a significant relationship between year and planting date, the trend among Illinois, Iowa, Indiana and Michigan was consistent for earlier planting dates. Corn breeding programs may facilitate this trend towards earlier planting dates by selecting for cold hardiness/tolerance and variable maturity rates. These traits along with an earlier planting dates would further exacerbate the asynchrony between corn and rootworm phenology.

Regions where corn is planted early and crop diversity is limited to corn and soybeans are at greater risk of larval injury to rotated corn (Fig. 1a). As suggested, females that emerge in a corn crop that is unattractive have a greater propensity to emigrate. The likelihood of selecting an oviposition site that will be rotated to corn the following year would be great in a landscape limited to corn and soybeans (Fig. 1a; 50% if dispersal is random). In contrast, if the corn phenology remains constant across regions with greater crop diversity (Fig. 1b) the probability that females would oviposit in a field rotated to corn the following year and likelihood of injury to rotated corn would decrease. Thus, spatial heterogeneity in a landscape could 'buffer' the impacts of phenology-mediated dispersal. Conversely, corn planted later relative to the surrounding crop may reduce immigration into soybeans (Fig. 1c). This, too, could have a buffering effect as females would remain in corn longer and oviposit more of their eggs in corn.

A trend in planting dates that occurred before the initial outbreak of injury to rotated corn in east-central Illinois may illustrate the potential 'buffering' interaction of landscape diversity and crop phenology. From 1983 to 1987, the average time to 50% anthesis occurred 20-days earlier in Illinois and Michigan (Fig. 2). If corn phenology is the driving factor behind rootworm dispersal from corn, then crop conditions in both states during 1983-1987 would facilitate oviposition in soybeans. Interestingly, at the end of this five-year period, first reported incidence of injury to rotated corn occurred in central Illinois (Levine and Oloumi-Sadeghi 1996). The absence of similar reports in Michigan may reveal the 'buffering' capacity provided by this state's greater farmland diversity.

CONCLUSION

In conclusion, research over the past eight years provides new information suggesting that the behavioral change associated with rotation resistant *D. v. virgifera* may not be due to a genetic variant, but rather can be explained by a corn phenology mediated dispersal. As corn matures and becomes a less attractive and acceptable food, *D. v. virgifera* dispersal increases. In areas of strict corn-soybean rotation, where corn is also planted with a high degree of synchrony, emigrating beetles will predominately encounter fields of equally unattractive corn or, alternatively, a soybean field (Fig. 1a). Given the potential for indiscriminate ovipositional behavior of *D. v. virgifera* and a lack of attraction to soybeans, adult emigration from cornfields may be responsible for oviposition in soybeans in the landscape described above. This an alternative explanation for rootworm injury to rotated corn does not require the existence of a rotation-resistant population, and may explain why differences between putative rotation-resistant and wild type populations have not been observed.

Whether a genetically distinct rotation-resistant population exists may not be as important as the affect crop diversity and corn phenology have on dispersal of *D. v. virgifera*. I have presented evidence that both of these features of the landscape affect rootworm behavior. By adjusting landscape structure to reduce emigration from cornfields, the effectiveness of crop rotation could be increased. Given the impact of planting date on rootworm population and oviposition (Musick et al. 1980, Hill and Mayo 1974, Naranjo and Sawyer 1988), this may be the most effective method to reduce immigration into soybean fields. For example, later planted border-rows may provide a 'barrier' to rootworms emigrating from corn (Fig. 1c, field in bottom right hand corner), significantly reducing their egg-laying in soybeans. Such a strategy may be compatible with resistance management practices for transgenic crops where delaying rootworm immigration from cornfields is desirable. Improving our understanding of how landscape structure influences rootworm immigration into soybeans may suggest improved management practices for rotated corn.

Table 1. Historical trend[a] in the timing of 50% corn planting and 50% anthesis from 1970 to 2000

State	*50% planting*			*50% anthesis*		
	Slope	r^2	P	Slope	r^2	P
Illinois	-0.03	0.04	0.28	-0.001	0.005	0.73
Indiana	*-0.03*	*0.04*	*0.31*	*-0.02*	*0.04*	*0.33*
Iowa	-0.03	0.06	0.21	0.01	0.01	0.64
Michigan	-0.02	0.03	0.34	-0.02	0.02	0.48
Nebraska	*-0.06*	*0.24*	*0.01*	*-0.03*	*0.13*	*0.09*

[a] Slope and r^2 values from linear regression analysis, df = 1, 27.

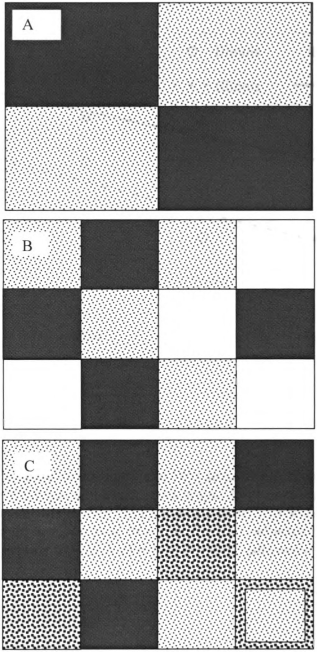

Figure 1. Comparison of two generalized landscapes of similar area, highlighting differences in field size, crop diversity and corn phenology that would (A) facilitate rootworm dispersal into soybean fields and (B) mitigate *D. v. virgifera* dispersal into soybean fields. Landscape C represents how adjusting corn phenology may reduce the rate of rootworm egg-laying in soybeans both on a regional level and field level (field in lower right hand corner). This field represents a cornfield with border rows planted later than the interior to produce a barrier of corn in a younger phenological stage. Shading represent crop types; black = soybean, small dots = corn post-anthesis, and large dots = corn in anthesis, white = neither corn or soybeans.

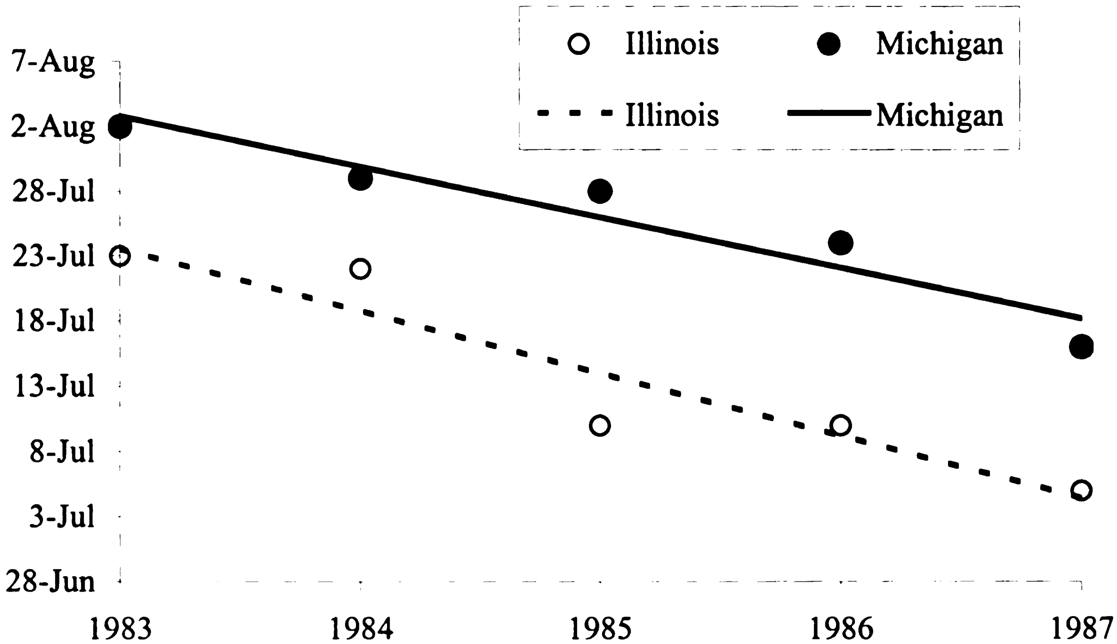

Figure 2. Temporal trends in time to 50% anthesis based on a state-wide averages for Michigan and Illinois corn crops. Data were summarized from USDA crop-progress reports. Lines indicate linear regression relationship for Michigan (solid line, $F = 32.8$, df = 1, 4; $P = 0.01$, adjusted $r^2 = 0.89$) and Illinois (dotted line, $F = 25.0$, df = 1, 4; $P = 0.02$, adjusted $r^2 = 0.86$).

APPENDICES

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2003-05

Title of thesis or dissertation (or other research projects):
Is corn phenology responsible for the failure of crop rotation to manage western corn rootworm (*Diabrotica virgifera virgifera* LeConte)?

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

NA

Investigator's Name(s) (typed)
Matthew E. O'Neal

Date March 28, 2003

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:
Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1
Voucher Specimen Data
Page 1 of 1 pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other
Diabrotica virgifera virgifera LeConte	USA, Nebraska, Saunders Co.					10		
	USA, Illinois, Champaign Co.					10		
	USA, Michigan, Ingham Co.					10		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Matthew E. O'Neal

Date March 28, 2003

Voucher No. 2003-05

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

REFERENCES CITED

Alchanatis, V., A. Navon, I. Glazer, and S. Levski. 2000. An image analysis system for measuring insect feeding effects caused by biopesticides. J. Agric. Eng. Res. 77: 289-296.

Angello, A. M. and C. W. MacMonegle, Jr. 1982. Insect situation and use of insecticides in 1981, pp. 104-122. *In* Proceedings of the 1982 Illinois Agricultural Pesticides Conference, January 6 and 7. Cooperative Extension Service, University of Illinois at Urbana-Champaign.

Anonymous. 1999. http://rsb.info.nih.gov/nih-image/

Anonymous. 2002. http://www.nass.usda.gov/indexcounty.htm.

Bergman, M.K., and F.T. Turpin. 1984. Impact of corn planting date on the population dynamics of corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 13: 898-901.

Bergman, M.K., and F.T. Turpin. 1986. Phenology of field populations of corn rootworm (Coleoptera: Chrysomelidae) relative to calendar date and heat units. Environ. Entomol. 15: 109-112.

Branson, T. F and J. L. Krysan. 1981. Feeding and oviposition behavior and life cycle strategies of Diabrotica: an evolutionary view with implications for pest management. Environ. Entomol. 10: 826-831.

Colwell, C. E. 1984. Insect situation and use of insecticides in 1983, pp. 104-122. *In* Proceedings of the 1984 Illinois Agricultural Pesticides Conference, January 4 and 5. Cooperative Extension Service, University of Illinois at Urbana-Champaign.

Darnell, S. J., L.J. Meinke, and L.J. Young. 2000. Influence of corn phenology on adult western corn rootworm (Coleoptera: Chrysomelidae) distribution. Environ. Entomol. 29: 587-595.

Davis, P.M., N. Brenes, and L.L. Allee. 1996. Temperature dependent models to predict regional differences in corn rootworm (Coleoptera: Chrysomelidae) Phenology. Environ. Entomol. 25: 767-775.

DiFonzo, C. D. 1998. Special Corn Rootworm Issue. Michigan State University Extension, Crop Advisory Team Alert, 13:12.

Elliott, N.C., R.D. Gustin, and S.L. Hanson. 1990. Influence of adult diet on the reproductive biology and survival of the western corn rootworm, *Diabrotica virgifera virgifera*. Entomol. Exp. Appl. 56: 15-21.

Elliott, N.C., J.J. Jackson, and R.D. Gustin. 1990. Predicting western corn rootworm beetle (Coleoptera: Chrysomelidae) emergence from the soil using soil or air temperature. Can. Ent. 122: 1079-1091.

Elliott, N.C., J.J. Jackson, G.R. Sutter, and D.A. Beck. 1991. A descriptive study of the population dynamics of adult Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae) in artificially infested cornfields. Great Lakes Entomol. 24: 159-167.

Fisher, J.R., G.R. Sutter, and T.F. Branson. 1990. Influence of corn planting date on the life stage development and phenology of Diabrotica virgifera virgifera. Entomol. Exp. Appl. 54: 219-224.

Fisher, J.R., G.R. Sutter, and T.F. Branson. 1991. Influence of corn planting date on the survival and on some reproductive parameters of Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). Environ. Entomol. 20: 185-189.

Gray, M. E. and J. J. Tollefson. 1988. Emergence of western and northern corn rootworms (Coleoptera: Chrysomelidae) from 4 tillage systems. J. Econ. Entomol. 81:1398-1403.

Godfrey, L. D., and F. T. Turpin. 1983. Comparison of western corn rootworm (Coleoptera: Chrysomelidae) adult populations and economic thresholds in first-year and continuous cornfields. J. Econ. Entomol. 76: 1028-1032.

Gonzalez, A.G., I. A. Jimenez, A. G. Ravelo, X. Belles, and M. D. Piulachs. 1992. Antifeedent activity of dihydro-B-agarofuran sesquiterpenes from Celastraceae against *Spodoptera littoralis*. Biochem. Syst. Ecol. 20: 311-315.

Gustin, R. D. 1979. Effect of two moisture and population levels on oviposition of the western corn rootworm. Environ. Entomol. 8: 406-407.

Hammack, L. 1997. Corn volatiles as attarctants for northern and western corn rootworm beetles (Coleoptera: Chrysomelidae: *Diabrotica* spp.). J. Chem. Ecol. 22: 1237-1254.

Hammond, R. B., L. G. Higley, L. P. Pedigo, L. Bledsoe, S. M. Spomer, and T. A. Degooyer. 2000. Simulated insect defoliation on soybean: influence of row width. J. Econ. Entomol. 93: 1429-1436.

Hein, G. L., and J. J. Tollefson. 1985. Seasonal oviposition of northern and western corn rootworms (Coleoptera: Chrysomelidae) in continuous cornfields. J. Econ. Entomol. 78: 1238-1241.

Hein, G.L. and J.J. Tollefson. 1987. Model of the biotic potential of western corn rootworm (Coleoptera: Chrysomelidae) adult populations, and its use in studying population dynamics. Environ. Entomol. 16: 446-452.

Hill, R. E. and Z B Mayo. 1974. Trap-corn to control corn rootworms. J. Econ. Entomol. 67: 748-750.

Hoy, C. W., and F. R. Hall. 1993. Feeding behavior of *Plutella xylostella* and *Leptinotarsa decemlineata* on leaves treated with *Bacillus thuringiensis* and esfenvalerate. Pestic. Sci. 38: 335-340.

Jansky, S., S. Austin-Phillips, and C. McCathy. 1999. Colorado potato beetle resistance in somatic hybrids of diploid interspecific *Solanum* clones. Hortscience. 34: 922-927.

Kirk, V. M., C. O. Calkins, F. J. Post. 1969. Oviposition preferences of western corn rootworms for various soil surface conditions. J. Econ. Entomol. 61:1322-1324.

Krysan, J. L. 1986. Introduction: biology, distribution, and identification of pest *Diabrotica*, pp. 1-23. *In* J. L. Krysan and T. A. Miller, eds. Methods for the study of pest *Diabrotica*. Springer-Verlag, New York.

Landis, D.A., E. Levine, M.J. Haas, and V. Meints. 1992. Detection of prolonged diapause of northern corn rootworm in Michigan (Coleoptera: Chrysomelidae). Great Lakes Entomol. 5: 215-222.

Levine, E. and M. E. Gray. 1996. First-year corn rootworm injury: east central Illinois research progress to date and recommendations for 1996, pp. 3-13. *In* Proceedings of the 1996 Illinois Agricultural Pesticides Conference, January 8 and 9. Cooperative Extension Service, University of Illinois at Urbana-Champaign.

Levine, E., and H. Oloumi-Sadeghi. 1991. Management of Diabroticite rootworms in corn. Annu. Rev. Entomol. 36: 229-255.

1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn grown for seed production following soybeans grown for seed production. J. Econ. Entomol. 89: 1010-1016.

Levine, E., J. L. Spencer, S. A. Isard, D. W. Onstad, and M. E. Grey. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am Entomol. 48: 94-107.

Lindow, S. E., and R. R. Webb. 1983. Quantification of foliar plant disease symptoms by microcomputer-digitized video image analysis. Phytopathol. 73: 520-524.

Landis, D.A., E. Levine, M.J. Haas, and V. Meints. 1992. Detection of prolonged diapause of northern corn rootworm in Michigan (Coleoptera: Chrysomelidae). Great Lakes Entomol. 5: 215-222.

Mitchell, F. L., and J. L. Lasswell. 2000. Digital dragonflies. Am. Entomol. 46: 110-116.

Metcalf, R. L. 1986. Plant volatiles as insect attractants. CRC Critical Reviews in Plant Sci. 5: 251-301.

Musick, G.J., H.C. Chiang, W.H. Luckmann, Z B Mayo, F.T. Turpin. 1980. Impact of planting dates of field corn on beetle emergence and damage by the western and the northern corn rootworms in the Corn Belt. Ann. Entomol. Soc. Am. 73: 207-215.

Naranjo, S. E. 1991. Movement of corn rootworm beetles, *Diabrotica* spp. (Coleoptera: Chrysomelidae), at cornfield boundaries in relation to sex, reproductive status and crop phenology. Environ. Entomol. 20: 230-240.

Naranjo, S. E. 1994. Flight orientation of *Diabrotica virgifera virgifera* and *D. Barberi* (Coleoptera: Chrysomelidae) at habitat interfaces. Annals of the Entomological Society of America 87: 383-394.

Naranjo, S. E. and A. J. Sawyer. 1988. A simulation model of northern corn rootworm, *Diabrotica barberi* Smith and Lawrence (Coleoptera: Chrysomelidae), in field corn. Environ. Entomol. 17:508-521.

Neild, R.J., and J.E. Newman. 1990. Growing season characteristics and requirements in the Corn Belt. National Corn Handbook. http://www.agcom.purdue.edu/AgCom/Pubs/NCH/NCH-40.html

O'Neal, M. E., C. DiFonzo, and D. Landis. 2002. Western corn rootworm (Coleoptera: Chrysomelidae) feeding on corn and soybean leaves affected by corn phenology. Environ. Entomol. 31: 285-292

O'Neal, M. E., M. E. Gray, and C. Smyth. 1999. Population characteristics of a western corn rootworm (Coleoptera: Chrysomelidae) strain in east-central Illinois corn and soybean fields. J. Econ. Entomol. 92: 1301-1310.

O'Neal, M. E., M. E. Gray, S. Ratcliffe, and K. L. Steffey. 2001. Predicting western corn rootworm (Coleoptera: Chrysomelidae) larval injury to rotated corn with Pherocon AM traps in soybeans. J. Econ. Entomol. 94: 98-104

Onstad, D. W., M. G. Joselyn, S. A. Isard, E. Levine, J. L. Spencer, L. W. Bledsoe, C. R. Edwards, C. D. DiFonzo, and H. Willson. 1999. Modeling the spread of western corn rootworm (Coleoptera: Chrysomelidae) populations adapting to soybean-corn rotation. Environ. Entomol. 28:188-194.

Onstad, D. W., J.L Spencer, C.A. Guse, E. Levine, and S.A. Isard. 2001. Modeling evolution of behavioral resistance by an insect to crop rotation. Entomol. Exp. Appl. 100: 195-201.

Onstad, D. W., D. W. Crowder, S. A. Isard, E. Levine, J. L. Spencer, M. E. O'Neal, S. T. Ratcliffe, M. E. Gray, L. W. Bledsoe, C. R. Edwards, C. D. DiFonzo, and B. Eisley. *In review*. Does landscape diversity slow the spread of rotation-resistant western corn rootworm (Coleoptera: Chrysomelidae).

Peterson, R.K.D., S. D. Danielson, and L. G. Higley. 1993. Yield responses of alfalfa to simulated alfalfa weevil injury and development of economic injury levels. Agron. J. 85: 595-601.

Rice, M. E. and J. Tollefson. 1999. Corn rootworms and lodged first-year corn. Iowa State University Extension, Integrated Crop Management. IC-482(22).

Ritchie, S. W., J. J. Hanway, and G. O. Benson. 1986. How a corn plant develops, special report 48. Iowa State University of Science and Technology Cooperative Extension Service, Ames, IA.

Ruppel, R.F. 1975. Dispersal of western corn rootworm, Diabrotica virgifera LeConte, in Michigan (Coleoptera: Chrysomelidae). J. Kans. Entomol. Soc. 48: 291- 296.

SAS Institute. 1996. SAS/STAT user's guide, version 6.12. SAS Institute, Cary, NC.

Sammons, A. E., C.R. Edwards, L. W. Bledsoe, P. J. Boeve, and J. J. Stuart. 1997. Behavioral and feeding assays reveal a western corn rootworm (Coleoptera:

Chrysomelidae) variant that is attracted to soybean. Environ. Entomol. 26: 1336-1342.

Schaafsma, A.W., G.H. Whitfield, and C.R. Ellis. 1991. A temperature-dependent model of egg development of the western corn rootworm, Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae). Can. Ent. 123: 1183-1197.

Shaw, J.K., J.H. Pallus, W.H. Luckmann. 1978. Corn rootworm oviposition in soybeans. J. Econ. Entomol. 71: 189-191.

Siegfried, B. D. and C. A. Mullin. 1990. Effects of alternative host plants on longevity, oviposition, and emergence of western and northern corn rootworms (Coleoptera: Chrysomelidae). Environ. Entomol. 19: 474-480.

Sifuentes, J. A. and R. H. Painter. 1964. Inheritance of resistance to western corn rootworm adults in field corn. J. Econ. Entomol. 57: 475-477.

Sinclair, J. B. and P. A. Backman [eds.]. 1989. Compendium of soybean diseases, 3rd edition. APS Press, St. Paul, MN.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry. W.H. Freeman and Company. New York.Spencer J. L., S. A. Isard, and E. Levine. 1999. Free flight of western corn rootworm (Coleoptera: Chrysomelidae) to corn and soybean plants in a walk-in wind tunnel. J. Econ. Entomol. 92: 146-155.

Stavisky, J. and P.M. Davis. 1997. The effects of corn maturity class on western corn rootworm (Coleoptera: Chrysomelidae) Phenology. Kans. Entomol. Soc. 70: 261-271.

Steffey, K.L. and K. D. Black. 1980. Insect situation and outlook and insecticide usage, pp. 124-149. *In* Proceedings of the 1980 Illinois Agricultural Pesticides

Conference, January 9 and 10. Cooperative Extension Service, University of Illinois at Urbana-Champaign.

Su, N. Y., and M. T. Messenger. 2000. Measuring wood consumption by subterranean termites (Isoptera: Rhinotermitidae) with digitized images. J. Econ. Entomol. 93: 412-414.

Wheeler, D. A., and M. B. Isman. 2001. Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. Entomol. Exp. Appl. 98: 9-16.

White, R. 1977. Sexual characters of species of *Diabrotica* (Chrysomelidae: Coleoptera). Ann. Entomol. Soc. Amer. 70: 168.

Wilde, G.E. 1971. Temperature effect on development of western corn rootworm eggs. J. Kans. Entomol. Soc. 44: 185-187.