Distribution of two rotation-resistant corn pests in eastern Iowa and effects of soybean varieties on biology of Diabrotica virgifera virgifera

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Distribution of two rotation-resistant corn pests in eastern Iowa and effects of soybean varieties on biology of *Diabrotica virgifera virgifera*

By

Michael Wilson Dunbar

A thesis submitted to the graduate faculty
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee
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Iowa State University
Ames, Iowa
2011

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Chapter 1. General Introduction

Thesis Organization

This thesis is organized into four chapters. Chapter 1 is a general introduction and literature review of *Diabrotica virgifera virgifera* LeConte and *Diabrotica barberi* Smith and Lawrence. Chapter 2 details a survey of eastern Iowa for abundance and distribution of *D. v. virgifera* and *D. barberi* as well as frequency of rotation-resistance in both species. Chapter 3 describes a laboratory study examining how soybean varieties affect *D. v. virgifera* survival and fecundity, particularly those soybean varieties with resistance to *Aphis glycines* Matsumura. Chapter 4 presents the general conclusions of this thesis.

Introduction

The western corn rootworm *Diabrotica virgifera virgifera* LeConte and the northern corn rootworm *Diabrotica barberi* Smith & Lawrence are two significant insect pests of corn in North America. Estimated costs of control and yield loss by these pests can reach $1 billion annually (Metcalf 1986). Both species have adapted to crop rotation in the Midwest; *D. barberi* through extended diapause and *D. v. virgifera* by ovipositing in non-corn crops. Knowledge of how rotation-resistance is distributed can help guide management strategies.

Objectives

Objectives of this thesis are the following:

1. Map the abundance and distribution of both *D. v. virgifera* and *D. barberi* throughout eastern Iowa from surveys conducted in 2008 and 2009.

2. Map the abundance and distribution of rotation-resistant *D. v. virgifera* throughout eastern Iowa from surveys conducted in 2008 and 2009.

3. Map the abundance and distribution of rotation-resistant *D. barberi* throughout eastern Iowa from a survey conducted in 2009.
4. Evaluate how varieties of soybeans, particularly those varieties with resistance to the soybean pest *A. glycines*, will affect the survival and fecundity of *D. v. virgifera*, and its consumption of soybean leaves.

**Literature Review**

*Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), the western corn rootworm, and *Diabrotica barberi* Smith and Lawrence (Coleoptera: Chrysomelidae), the northern corn rootworm, are both major pests of corn in North America. Both species have been pests of corn for over 100 years (Forbes 1882, Gillette 1912). Soil and aerial insecticide applications combined with yield loss can cost $1 billion annually in the United States (Metcalf 1986).

**General Biology.** Both *D. v. virgifera* and *D. barberi* are univoltine and primarily consume corn tissue throughout their life history (Chiang 1973). Adults are present in corn fields from late June until frost (Spencer et al. 2009). Eggs are oviposited in soil during late summer (Hein and Tollefson 1985b) where they will enter diapause throughout the winter (Chiang 1973). Oviposition in both species occurs primarily in corn fields (Spencer and Levine 2008). Experiments conducted in the field constructing life-tables reported that 46% of diapausing eggs died during overwintering (Toepfer and Kuhlmann 2006), most likely due to desiccation and low temperatures (Calkins and Kirk 1969).

Larvae of both species will hatch in the spring and are generally thought to be functionally monophagous corn root feeders (Krysan 1993). Recent evidence has shown that *D. v. virgifera* is capable of complete development on a number of grass species (Clark and Hibbard 2004, Wilson and Hibbard 2004). In comparisons performed in greenhouse assays 14 of 23 artificially infested plant species had adult emergence (Oyediran et al. 2004). Levels of emergence were not significantly different between corn and pubescent wheatgrass (Oyediran et al. 2004). Studies have shown that *D. barberi* is also capable of complete development on other grass species besides corn (Branson and
Ortman 1971). In similar greenhouse assays performed with *D. v. virgifera*, *D. barberi* completed development and emerged from only 6 of 27 species of prairie grasses tested (Oyediran et al. 2008).

Hatched larvae orient towards corn root violates such as carbon dioxide (Bernklau and Bjostad 1998), though high mortality of early instars is probably due to larvae failing to locate root masses (Oloumi-Sadeghi and Levine 1989, Toepfer and Kuhlmann 2006). Feeding from larvae can injure corn roots, which may lead to reduced nutrient and water uptake (Kahler et al. 1985). Root injury also makes corn more susceptible to lodging and yield loss (Spike and Tollefson 1991). Lodged plants can reduce yield by complicating mechanical harvest (Riedell 1990). Pupation occurs after three larval instars near the soil surface and for *D. v. virgifera* can last between 5 and 10 days (Fisher 1986).

Male *D. v. virgifera* will emerge before females. Following emergence, females will adapt a call posture and release a sex pheromone to attract males. However, the calling posture is not necessary for males to locate females (Hammack 1995). Females are thought to mate very quickly, 96% of females observed mating were suspected of emerging within 24h (Quiring and Timmins 1990). Males can mate with multiple females, though the majority of females only mate once (Branson et al. 1977).

Female *D. v. virgifera* will begin to oviposit within 6-10 days and will continue to produce eggs for 4-5 weeks after mating (Sherwood and Levine 1997). Preovipositional periods in *D. barberi* are reported between 11 and 14 days for temperatures ranging between 25°-30°C (Naranjo and Sawyer 1987). The two species vary widely in reproductive capability. Mean fecundity of *D. barberi* in a laboratory setting was 274 eggs laid in an average of 8.7 clutches every 6.4 days (Naranjo and Sawyer 1987). Under similar laboratory conditions *D. v. virgifera* had a mean fecundity of 1087 eggs produced by an average of 13 clutches every 5 days (Hill 1975).

Adults of both species most commonly feed on corn tissue such as corn silks, kernels, and tassels. Pollen from plants other than corn are more commonly consumed by *D. barberi*, which can
also be found more frequently outside of corn fields than *D. v. virgifera* (Hill and Mayo 1980). Short-range flight observations of both species indicated that movement within homogenous surrounding such as a corn field is random, but movement at the boundary between heterogeneous surroundings (two corn fields at differing phenologic states or two different types of crops) is non random (Naranjo 1994). Colonization of first-year corn by *D. v. virgifera* will occur within a week after emergence and populations within these fields are female-biased (Godfrey and Turpin 1983). Female-biased dispersion also occurs with *D. barberi* as females are found in higher proportion in late flowering corn fields (Naranjo and Sawyer 1988).

**Measuring Injury from *Diabrotica* spp.** Several scales have been created to measure root injury from *Diabrotica* spp. Two such scales are the Iowa 1-6 scale (Hills and Peters 1971) and 1-9 scale (Musick and Suttle 1972). The Iowa 1-6 scale was the more commonly used of the two (Tollefson 2007). Both require that roots be removed from the ground, the above ground growth be removed, and soil around the roots to be washed off. A rating of 1 on both scales indicates little to no feeding while 6 and 9 represent the most severe injury. A root injury rating of 2.5 on the Iowa 1-6 scale represents the economic threshold (Turpin et al. 1972) and its equivalent on the 1-9 scale is 3.6 (Foster et al. 1986). A new scale called the node-injury scale developed by Oleson et al. (2005) which was quantitative rather than categorical and runs from 0-3.

Predicting injury from *Diabrotica* spp. abundance is complicated. Peter (1963) calculated an economic threshold of 10 larvae per plant, though artificial infestations of 300 *D. v. virgifera* and *D. barberi* eggs recorded root injury ratings of 2.5 and 2.9 on the Iowa 1-6 scale respectively (Fisher 1985). Using the regression lines from the plot of root damage rating vs infestation rate as calculated by Fisher (1985), it would take 70 and 240 artificially infested eggs to produce a root injury rating of 2.5 on the Iowa 1-6 scale for *D. v. virgifera* and *D. barberi* respectively. Measures of whole-plant adult counts determined that 1.62 adults per plant in continuous corn and 0.71 adults per plant for first-year corn would reach economic thresholds of 2.5 on the Iowa 1-6 scale (Godfrey and Turpin
Hein and Tollefson (1985a) created an estimation of adult populations for economic threshold predicting injury to corn the following year. An array of 12 unbaited Pherocon AM sticky traps are distributed throughout a corn field during the last three weeks of August, and these traps should be changed every 7 days. If average Diabrotica spp. captured is greater than 6 adults per sticky trap per day then the field risks injury the following year (Hein and Tollefson 1985a).

**Control Tactics.** Chemical control of *Diabrotica* spp. began in the 1940’s with soil surface applications of benzene hexachloride (Hill et al. 1948). Both *D. v. virgifera* and *D. barberi* prove difficult to manage as resistance to chemical control became apparent over time. Efficacy of chlorinated hydrocarbons lasted for two decades (Levine and Ololumi-Sadeghi 1991) as resistance was found in Nebraska, South Dakota, and Kansas (Ball and Weekman 1962, 1963). Rapid range expansion during this era by *D. v. virgifera* and *D. barberi* occurred as a result of this resistance to chemical control (Metcalf 1983). Evidence of *D. v. virgifera* resistance to methyl parathion and carbaryl was later found in populations from Nebraska (Meinke et al. 1998). Resistance to carbaryl was also found in *D. v. virgifera* populations around north-central Kansas (Zhu et al. 2001).

Host plant resistance can be a valuable pest-management strategy. Traditionally host plant resistance has been classified into three main categories - nonpreference, antibiosis, and tolerance (Painter 1951). Nonpreference was later changed to antixenosis because antixenosis better defines a plant characteristic as opposed to a pest’s reaction (Kogen and Ortman 1978). The search for host plant resistance to *Diabrotica* spp. in corn began in 1930 (Bigger et al. 1938). Root injury ratings from field trials were concluded to be the clearest method of evaluating resistance to *D. v. virgifera* (Knutson et al. 1999), though other studies have argued that larger root size and secondary root regrowth are better correlated with yield than root-injury rating (Tollefson 2007). Evaluation of corn hybrids commonly used in the 1980’s suggested that tolerance through large root systems was prevalent in corn genotypes of that time (Riedell and Evenson 1993). Resistance other than tolerance was found in the early 1980’s, but whether by antibiosis or antixenosis remained unclear (Branson et
Antibiosis has been found in the form of hydroxamic acids in root tissue (Assabgui et al. 1995a, 1995b). In field trials survival of *D. v. virgifera* was inversely related to the amount of hydroxamic acid found in corn roots (Assabgui et al. 1995a). The first two corn lines shown to have strong antibiosis resistance, through reduced plant injury and adult recovery, without being genetically modified were recently discovered (Khishen et al. 2009).

In 2003 commercially available transgenic rootworm-protected corn hybrids with single traits expressing *Bacillus thuringiensis* (Bt) proteins were introduced (United States Environmental Protection Agency 2009). Use of Bt corn has reduced application of insecticides against *Diabrotica* spp. by more than 75% (United States Environmental Protection Agency 2009). Bt is indigenous to multiple environments (Bernhard et al. 1997) including soil and insects (Carozzi et al. 1991). The mechanism of action for Bt proteins begins as the insect consumes the toxin, the toxin then binds with midgut receptors, which is followed by insertion of the toxin into the apical membrane creating pores (Schnepf et al. 1998). Bt proteins in corn root tissue will kill larvae that feed on the corn plant (Vaughn et al. 2005). Early assays of neonate *D. v. virgifera* found decreased feeding on roots expressing protein Cry3Bb1 compared susceptible lines (Clark et al. 2006). Rootworm-protected pyramid hybrids, those with multiple traits expressing Bt proteins, was registered in 2009 (United States Environmental Protection Agency 2009). Models have shown that multiple trait transgenic corn can delay resistance evolution for longer than hybrids with only one trait (Onstad and Meinke 2010). Future hybrids may include RNA interference complimenting Bt proteins for protection against *D. v. virgifera* and *D. barberi* (Baum et al. 2007).

Crop rotation has been recommended for control of both species for over a century (Krysan 1993, Spencer and Levine 2008). Crop rotation functions best when a pest has a life stage that is relatively immobile, requires consumption of host plant for development, and are unable to survive for extended times without a host (Spencer and Levine 2008). Larvae of *D. v. virgifera* and *D. barberi* fit comfortably into all three of these requirements as they are functionally monophagous corn
feeders, movement in the soil is thought to range between 60 and 100 cm maximum (Short and Luedtke 1970, Hibbard et al. 2004), and larvae will starve quickly without the presence of a suitable host. A two-year rotation of corn and soybeans can increase yields by 5 to 20% compared with corn in continuous production (Bullock 1992). Yield advantages gained by two-year rotation of corn and soybeans remain even when increased amounts of fertilizer and pesticide are applied to continuous corn production (Bullock 1992). Crop rotation is also economically advantageous as it does not have technology fees associated with Bt corn (Martin and Hyde 2001, Hyde et al. 2003). Environmental and public health costs, particularly those associated with chemical control (Pimental et al. 1993), are reduced with crop rotation. However, despite its effectiveness, both *D. v. virgifera* and *D. barberi* have both adapted to crop rotation.

**Rotation-Resistant *D. barberi***. Injury to first-year corn by *D. barberi* has been observed as early as the 1920’s (Bigger 1932). Observations of *D. barberi* eggs placed in the soil found that 2% were capable of surviving through two winters (Chiang 1965). That *D. barberi* were the dominate species in regions where crop rotation was widely practiced and that they were more frequently observed in non-corn fields (Hill and Mayo 1980) led to the hypothesis that *D. barberi* oviposition is linked with adult feeding (Branson and Krysan 1981). In a second experiment placing *D. barberi* eggs in soil found that egg hatch followed a bimodal distribution over a two year period, and that 40% of eggs hatched after two winters (Krysan et al. 1984). Eggs hatching after two winters represented a high enough percentage of hatch to cause as much economic concern to corn fields as the proportion of eggs that hatched after one winter. In follow up field studies Krysan et al. (1986) observed 40-47% of *D. barberi* eggs from areas where crop rotation was practiced expressed extended diapause as opposed to only 8% from areas where continuous corn was practiced. Levine et al. (1992) found evidence of diapause in populations lasting from 1 to 4 years with the percentage of eggs hatch per year mimicking crop rotation patterns used from each population’s origin. If rotation-resistant *D. barberi* are exposed to a consistent pattern of crop rotation over long periods of time the
risk of economically damaging populations increase (Fisher et al. 1994). Further, it is suggested that rotation-resistant *D. barberi* may be able to adapt to practices of individual corn producers (Krysan 1993).

Traditional and extended diapause *D. barberi* phenotypes occur together and studies of gene flow between them have found no evidence of reproductive isolation between traditional *D. barberi* and those expressing extended diapause (Krafsur 1995). Rotation-resistant *D. barberi* are at a reproductive disadvantage when corn is grown continuously because their reproductive rate is halved, while traditional *D. barberi* are at a disadvantage when corn is regularly rotated (Spencer and Levine 2008). Because mating between traditional and extended diapause *D. barberi* is essentially random, expression of extended diapause will only slowly increase in frequency and only where crop rotation is practiced more frequently (Krafsur 1995). The range of rotation-resistance in *D. barberi* extends throughout much of the northern Corn Belt with reports of extended diapause in Iowa, Illinois, Michigan, Minnesota, Nebraska, South Dakota, and North Dakota (Foster 1987, Landis et al. 1992, Levine et al. 1992, Krysan 1993), though in some states rotation-resistance is not often present at economically damaging levels (Steffey et al. 1992).

**Rotation-Resistant *D. v. virgifera***. The first observed damage to rotated corn by *D. v. virgifera* occurred in east-central Illinois in 1987 (Gray et al. 1996, Gray et al. 2009). In 1995 the first reported severe damage by *D. v. virgifera* to annually rotated corn occurred in the same area (Gray et al. 1996). Sixteen first-year corn fields sampled by Gray et al. (1996) averaged damage of 3.72 on the 1-6 Iowa State University root-rating scale (Hills and Peters 1971). Root ratings of 4 indicate one node of root is completely destroyed and that economic loss is possible.

The mechanism of how *D. v. virgifera* were circumventing crop rotation was unknown and many hypotheses emerged. Laboratory tests found that *D. v. virgifera* females were repelled from corn pots by pyrethoroids into soybean pots (Levine and Oloumin-Sadeghi 1996). This lead to the hypothesis that pyrethoroid treated corn would repel *D. v. virgifera* into untreated fields which were
primarily soybean. This hypothesis was quickly dismissed as rotation resistance spread outward from a suspected epicenter (Onstad et al. 1999). Another hypothesis was that *D. v. virgifera* eggs were expressing prolonged diapause. Prolonged diapause in *D. v. virgifera* had previously been investigated and results yield no evidence of the trait (Krysan et al. 1984). Further studies eventually found expression of prolonged diapause in *D. v. virgifera*, but extended diapause was still ruled out as the cause since only 0.14% and 0.21% of eggs from problem and non-problem areas, respectively, underwent prolonged diapause (Levine et al. 1992).

In a 6 choice preference assay *D. v. virgifera* from areas where rotation-resistance was prevalent were found to prefer soybean without crop residue and pollinated corn more than water wicks, corn residue, pollinating corn, or soybean with corn residue (Sammons et al. 1997). When *D. v. virgifera* from rotation-susceptible areas were tested in the same assay they preferred pollinating and pollinated corn, and results indicated that rotation-resistant *D. v. virgifera* were attracted to soybean (Sammons et al. 1997). This hypothesis was later refuted by two studies. In a series of free flight experiments *D. v. virgifera* were allowed to move throughout a wind tunnel filled with varying arrangements of corn and soybeans and no matter how the plant types were configured *D. v. virgifera* were more commonly found on corn (Spencer et al. 1999). The results of this study remained consistent regardless of whether *D. v. virgifera* used were captured from rotation-resistant affected areas or from without. Spencer et al. (1999) further argued that decreased adult fidelity to corn or increased tendencies to leave corn were more constant with what was known about rotation-resistance.

When electroantennogram responses to corn and soybean volatiles from populations of rotation-resistant and rotation-susceptible of *D. v. virgifera* were tested no differences were discovered between treatments and population location (Hibbard et al. 2002). Female rotation-resistant *D. v. virgifera* were more responsive overall than rotation-susceptible females. This supported the argument that rotation-resistant *D. v. virgifera* may be leaving corn fields because they have a broader range of olfactory stimuli (Hibbard et al. 2002). In later behavioral assays females from areas with
rotation-resistance were found to be more active than those from rotation-susceptible areas (Knolhoff et al. 2006).

Surveys of corn and soybean fields in rotation-resistant problem areas recorded higher capture of *D. v. virgifera* on Pherocon AM and vile traps in soybean fields than corn fields (O’Neal et al. 1999). O’Neal et al. (2001) found that 27% of the variation in root injury to rotated corn could be explained by *D. v. virgifera* densities in soybean fields the previous year. Hypotheses that *D. v. virgifera* has circumvented crop rotation through a behavioral adaption by ovipositing eggs in soybean fields started to become more clear (Levine et al. 2002). An economic threshold of 4.7 adults captured per Pherocon AM sticky trap per day in soybean fields using techniques similar to those described by Hein and Tollefson (1985a) would predict root injury ratings of 3 on the Iowa 1-6 scale if that field was rotated to corn the following year (O’Neal et al. 2001). Relaxed fidelity to corn field oviposition by rotation-resistant *D. v. virgifera* is not limited to soybean fields but can also occur in oats, alfalfa, and winter wheat (Rondon and Gray 2004, Schroeder et al. 2005). However, oviposition occurs at higher frequency in soybean fields because soybean is the most common crop besides corn in areas affected by rotation resistance (Onstad et al. 2003).

Rotation-resistance has continued to spread outward from the epicenter in east-central Illinois and models have since been used to predict the expansion of rotation-resistant *D. v. virgifera* range. Simple models of the spread of rotation-resistance have been accurately created and postulated that the trait increased in range at a rate of 10-30km/yr between 1986 and 1997 (Onstad et al. 1999). The speed of rotation-resistant range expansion was best modeled using prevailing wind and storm directions, and that rotation-resistance would spread faster eastward because of these weather patterns. Models accounting for landscape heterogeneity were also created and showed that as heterogeneity in the landscape increased the spread of rotation-resistance decreased (Onstad et al. 2003).
**Interaction between *D. v. virgifera* and *D. barberi.*** Interaction between *D. v. virgifera* and *D. barberi* is likely as both species exist in the same habitat (Chiang 1973). The competitive exclusion principle states that species that occupy the same niche cannot do so indefinitely (Hardin 1960). If species are studied closely then they will invariably occupy unique niches, though competitive exclusion still exists (Rockwood, 2006). Competitive exclusion does not always occur when species use similar resources. No evidence of competition was found when 13 different tropical Coleopteran species that all used similar food and habitat were studied (Strong 1982). Environments that are subject to regular catastrophic events that destroy old and create new habitats will allow competing species to coexist (Hutchinson 1953). Habitat for *D. v. virgifera* and *D. barberi* is constantly being demolished and recreated as corn fields are rotated to and from corn while resources are generally unlimited.

In persistence trials of the soil insecticide aldrin, limited evidence of *D. barberi* suppressing *D. v. virgifera* populations was observed (Chiang and Raros 1968). In corn strips that were left untreated with insecticide had large populations of *D. barberi* and relatively small *D. v. virgifera* populations, but when aldrin residue limited the abundance of *D. barberi* the *D. v. virgifera* populations doubled. Larger body size was thought to make *D. v. virgifera* an inferior competitor because it would require more resources than *D. barberi* to survive (Chiang and Raros 1968).

Distribution of *Diabrotica* spp. in 1948 throughout Nebraska showed that *D. barberi* comprised 70% of all *Diabrotica* spp. captured (Hill and Mayo 1980). Annual surveys from 1960-1967 and field studies from 1967-1973 found that *D. v. virgifera* had displaced *D. barberi* as the dominate species (Hill and Mayo 1980). In areas where continuous corn was planted more frequently *D. v. virgifera* were more prevalent, but in areas where crop rotation was regularly practiced *D. barberi* was the more abundant species. This higher abundance of *D. barberi* in areas where crop rotation was more frequently practiced was originally attributed to oviposition in non-corn crops (Hill and Mayo 1980), which is now know to be caused by extended diapause.
When *D. v. virgifera* and *D. barberi* were infested in pots of corn grown in the greenhouse at varying densities *D. v. virgifera* would reduce survival of larval *D. barberi* (Piedrahita et al. 1985). Larval *D. barberi* were also spatially displaced by *D. v. virgifera*, when *D. v. virgifera* were absent *D. barberi* larvae were found throughout the infested pots. When *D. v. virgifera* were present *D. barberi* larvae were found around the edge of pots. While Piedrahita et al. (1985) found that *D. v. virgifera* were unaffected by the presence of *D. barberi* in mixed infestations similar investigations did not completely agree with this conclusion. In greenhouse infestation experiments *D. v. virgifera* would reduce survival of larval *D. barberi*, but it was also found that *D. barberi* would reduce survival of larval *D. v. virgifera* (Woodson 1993). In a second greenhouse study Woodson (1994) found that *D. v. virgifera* emergence was affected more by increasing *D. v. virgifera* infesting densities than increasing *D. barberi* infesting densities. Female *D. v. virgifera* emergence was reduced as *D. barberi* infesting densities increased. Emergence of *D. barberi* was negatively affected by both increasing *D. v. virgifera* and *D. barberi* infesting densities. Conversely, survey of *D. v. virgifera* and *D. barberi* distributions in southern Quebec, Canada corn fields found an absence of a strong interaction between the two species when species abundance was analyzed (Meloche et al. 2005).

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Chapter 2. Abundance and Distribution for Two Species of Corn Rootworm (Diabrotica spp.) and Prevalence of Rotation Resistance in Eastern Iowa

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Abstract

The western corn rootworm Diabrotica virgifera virgifera LeConte and the northern corn rootworm Diabrotica barberi Smith & Lawrence are two major pests of corn in North America. Both species have adapted to crop rotation in the Midwest, D. barberi through extended diapause and D. v. virgifera by ovipositing in non-corn crops. Surveys were conducted during 2008 and 2009 collecting data on the abundance of D. barberi and D. v. virgifera and included monitoring of the prevalence of rotation-resistant D. barberi and D. v. virgifera in eastern Iowa. In 2008 Pherocon AM sticky traps were placed in corn fields to measure abundance of both species and were also placed in soybean fields to monitor for rotation-resistant D. v. virgifera. Surveys were expanded in 2009 to include monitoring for rotation-resistant D. barberi and D. v. virgifera with emergence cages in first-year corn fields. Abundance of D. barberi and D. v. virgifera in corn fields was positively correlated, though fields were typically dominated by one species and field dominance by species appeared spatially segregated. Capture of D. v. virgifera in soybean fields most commonly occurred in northeastern Iowa, but emergence from first-year corn was rare. Rotation-resistant D. barberi emerged from first-year corn throughout eastern Iowa, the majority emerging in regions nearly matching historical distributions in Iowa. Range expansion of rotation resistant D. barberi has progressed slowly. Models created predicting the spread of rotation-resistant D. v. virgifera appear accurate, though the behavior appears not to have fully established itself in Iowa as of 2009.

Introduction
*Diabrotica virgifera virgifera* LeConte, the western corn rootworm, and *Diabrotica barberi* Smith and Lawrence, the northern corn rootworm, are both important pests of corn in North America. Control measures and yield loss from these pests is estimated to cost greater than $1 billion annually in the United States (Metcalf 1986). Both *D. v. virgifera* and *D. barberi* are univoltine species that primarily consume corn tissue throughout their life cycles (Chiang 1973). Oviposition occurs during late summer and early fall, primarily in corn fields. Larvae that hatch in the spring will develop on corn root tissue, but roots of other grass species can sustain larval growth (Branson and Ortman 1971, Oyediran et al. 2004, 2008). Root feeding can inhibit moisture and nutrient uptake as well as cause plant lodging and yield loss (Elliott et al. 1991, Spike and Tollefson 1991). Lodged plants can further reduce yield by complicating mechanical harvest (Riedell 1990). Larvae pupate in the soil and emerge as adults throughout midsummer (Levine and Oloomi-Sadeghi 1991).

Chemical control of *Diabrotica* spp. began in the 1940s with soil surface applications of benzene hexachloride (Hill et al. 1948). Both *D. v. virgifera* and *D. barberi* proved difficult to manage as resistance to chemical control became apparent over time. Efficacy of chlorinated hydrocarbons lasted for two decades before resistance was recorded (Ball and Weekman 1962, 1963). Resistance to methyl parathion and carbaryl would soon follow (Meinke et al. 1998, Zhu et al. 2001).

In 2003 transgenic rootworm-protected corn hybrids with single traits expressing *Bacillus thuringiensis* (Bt) proteins became commercially available. Bt proteins in corn root tissue will kill larvae that feed on the corn plant (Vaughn et al. 2005). Rootworm-protected pyramid hybrids, those with multiple traits expressing Bt proteins, were registered in 2009 (United States Environmental Protection Agency 2009). Models have shown that multiple trait transgenic corn can delay resistance evolution for longer than hybrids with only one trait (Onstad and Meinke 2010). Future hybrids may include RNA interference complimenting Bt proteins for protection against *D. v. virgifera* and *D. barberi* (Baum et al. 2007).
Host plant resistance, defined as antibiosis, antixenosis, and tolerance (Painter 1951, Kogen and Ortman 1978), can provide corn producers with an alternative to chemical control or genetically modified crops. The search for host plant resistance to *Diabrotica* spp. began in the 1930’s (Bigger et al. 1938) and continues today (Gray and Steffey 1998, Hibbard et al. 1999, Prischmann et al. 2009). No commercial hybrids produced today are resistant to *Diabrotica* spp. that are not genetically modified (Khishen et al. 2009). Earlier research has found tolerance in the form of large root systems or root regeneration (Owens et al. 1974, Branson et al. 1980). More recently, lines with varying degrees of antibiosis have been found (Assabgui et al. 1995, Khishen et al. 2009).

Crop rotation has been a recommended control of both *D. v. virgifera* and *D. barberi* for over a century (Spencer and Levine 2008). Eggs that have been oviposited in corn fields are immobile. When fields are rotated to a non-corn crop, larvae that hatch the following spring will starve because they cannot find a suitable host. Two-year corn soybean rotation can produce yields 5 to 20% greater than corn in continuous production and these advantages in yield remain even when increased levels of fertilizer and pesticide are applied to continuous corn (Bullock 1992). Other economic incentives of crop rotation include the absence of technology fees like those associated with Bt corn (Martin and Hyde 2001, Hyde et al. 2003). Also, environmental and public health costs associated with chemical control are avoided with crop rotation (Pimental et al. 1993). While crop rotation is advantageous to corn producers by increasing yields and controlling *D. v. virgifera* and *D. barberi*, both species have adapted to rotated cropping systems.

Observed injury to first-year corn by *D. barberi* was first recorded in the 1920s (Bigger 1932). In several field studies eggs of *D. barberi* were found capable if surviving multiple winters and in some cases greater than 40% of egg hatch occurred after the second winter (Chiang 1965, Krysan et al. 1984, 1986). Eggs hatching after two winters are rotation-resistant through the physiological adaptation of extended diapause. Further research discovered that extended diapause could last up to 4 years and percentages of egg hatch per year would mimic rotation patterns from the
sampled population’s origins (Levine et al. 1992). The range of rotation-resistant *D. barberi* extends over much of the northern Corn Belt (Foster 1987, Landis et al. 1992, Levine et al. 1992, Krysan 1993), though in some areas expression of extended diapause occurs less frequently (Krysan et al. 1986, Steffey et al. 1992). Mating between rotation-susceptible and rotation-resistant *D. barberi* occurs randomly so expression of extended diapause will increase slowly and only in areas where crop rotation is frequently practiced (Krafsur 1995).

*Diabrotica virgifera virgifera* has also adapted to crop rotation but by a completely different mechanism. While *D. barberi* more readily feed on and disperse to non-corn agriculture than *D. v. virgifera* (Ludwig and Hill 1975, Hill and Mayo 1980), the majority of *D. barberi* oviposition still occurs in corn fields (Boetel et al. 1992). This is not true of rotation-resistant *D. v. virgifera* which has adapted to crop rotation by decreased oviposition fidelity to corn (Levine et al. 2002). Injury to first-year corn by *D. v. virgifera* began in the late 1980s and early 1990s (Gray et al. 1996). Soybean fields in areas experiencing rotation-resistance by *D. v. virgifera* recorded higher percentages of female *D. v. virgifera* than in corn fields (O’Neal et al. 1999) and 27% of the variation in root injury to first-year corn could be explained by the abundance of *D. v. virgifera* in soybean fields (O’Neal et al. 2001). Tests using free flight wind tunnel activity of *D. v. virgifera* (Spencer et al. 1999b) and *D. v. virgifera* electroantennogram responses to corn and soybean violates (Hibbard et al. 2002) found that rotation-resistant *D. v. virgifera* were not attracted to soybean. It is also clear that rotation-resistance does not convey any ability to gain sustenance from soybean feeding in *D. v. virgifera*, as survival of adults fed soybean was similar to adults that only had access to water (Mabry and Spencer 2003). Several studies observing movement and oviposition found that *D. v. virgifera* oviposition is indiscriminate and eggs could be found in many types of crops (Rondon and Gray 2003, 2004, Schroeder et al. 2005).

Both adaptations to crop rotation are not unique. Many insects are known to express extended diapause (Waldbauer 1978). Examples include *Neodiprion sertier* Geoffroy (Hymenoptera:
Diprionodae) that can diapause as an egg or prepupa for two or more years (Sullivan and Wallace 1967) and *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) which can overwinter as a pupa in soils for one or two winters (Oatman 1964). Behavioral adaptations similar to reduced ovipositional fidelity in *D. v. virgifera* have also been observed in *Plutella xylostella* L. (Lepidoptera: Plutellidae). Insecticide-resistant *P. xylostella* oviposit significantly more eggs around the host plant’s soil-stem interface than the more traditional location of the host leaf, suggesting a behavioral resistance to foliar-applied insecticide (Sarfraz et al. 2005).

Interaction between *D. barberi* and *D. v. virgifera* should exist since both share the same habitat and resources (Chiang 1973). Field observations have shown *D. v. virgifera* populations that increased when more abundant *D. barberi* population decreased, which led to the hypothesis that *D. v. virgifera* might be an inferior competitor due to its larger body size compared to *D. barberi* (Chiang and Raros 1968). Annual surveys from Nebraska corn fields between 1960 and 1967 demonstrated the opposite: *D. v. virgifera* displaced *D. barberi* in areas where corn was more frequently in continuous production (Hill and Mayo 1980). When corn plants in a greenhouse were artificially infested with varying densities of *D. barberi* and *D. v. virgifera* eggs, survival of both species decreased as the density of other species increased (Woodson 1993). Other studies have shown that interaction might be limited. Surveys of *D. barberi* and *D. v. virgifera* abundance in corn fields throughout Quebec, Canada found little evidence of interaction between species (Meloche et al. 2005).

Historical data on the distribution of rotation-resistant *D. barberi* indicates that extended diapause occurs mainly in northwest Iowa (Foster 1987). Models of the expansion of rotation-resistant *D. v. virgifera* predict this behavior to invade eastern Iowa sometime between 2007 and 2011 (Onstad et al. 1999, Levine et al. 2002). Eastern Iowa presents an opportunity to observe distribution and possible range expansion of both rotation-resistant adaptations. Abundance and distribution of *D. barberi* and *D. v. virgifera* throughout eastern Iowa was monitored in 2008 and 2009. Particular
focus was on prevalence rotation-resistant *D. v. virgifera* in 2008 and prevalence of rotation-resistance in both species in 2009. Estimating the distributions of these corn pests may help future pest management.

**Materials and Methods**

**2008.** Three transects were established in eastern Iowa to monitor for abundance of *D. barberi* and *D. v. virgifera* and the abundance and distribution of rotation-resistant *D. v. virgifera*. Transects were established parallel to 3 east-west highways in eastern Iowa averaging 235 ± 31.9 km in length and separated on average by 83 ± 6.6 km (Fig. 1). Transects were designated north, central, and south respective of their position in the state. Field sites used to monitor *Diabrotica* spp. were identified by Iowa State University regional agronomists and local cooperators. Two field sites were established within each county intersected by transects (Table 1). Each field site was composed of a rotated corn field and an adjacent soybean field for a total of 38 field sites containing 76 individually monitored fields in 2008. Sampling point distance from the transects averaged 1.14 ± 1.9 km and were separated on average by 19.05 ± 10.1 km.

Twelve unbaited Pherocon AM yellow sticky traps (Great Lakes IMP, Vestaburg, MI) were placed in corn and soybean fields beginning the last week of July in a manner similar to methods described by Hein and Tollefson (1985). Sticky traps were changed weekly for three weeks (28 July-1 Aug.; 4 Aug.- 8 Aug.; 11 Aug.-18 Aug.). In mid August traps were removed from corn fields, however monitoring continued in soybean fields for another two weeks (29 Aug.- 31 Aug.; 5 Sept.-7 Sept.) as an attempt to capture any delayed adult movement.

**2009.** Two transects were established in eastern Iowa to monitor for abundance of *D. barberi* and *D. v. virgifera* as well as abundance and distribution of rotation-resistance of both species (Fig. 1). Transects were established parallel to 2 north-south highways in eastern Iowa and averaged 278 ± 11.9 km in length and were separated on 108.8 ± 39.8 km. Transects were designated as east and west respectively. Field sites were again identified by Iowa State University regional agronomists
and local cooperators. Each field site was composed of a first-year non-corn rootworm Bt corn field, a rotated soybean field, and 1 to 2 continuous corn fields. No first-year corn fields used in this study were reported to have problems with volunteer corn the previous year when planted to soybean. Continuous corn fields were defined as any field in which corn had been grown for 2 or more consecutive years. All field types found at any given field site were located within a $2.0 \pm 1.6 \text{ km}$ radius. There were a total of 17 sampling locations (Table 2) containing a total of 57 individual fields monitored in 2009.

Six Illinois style emergence cages were placed in first-year corn fields during the last week of June. Emergence cages were modified from designs used in Musick and Fairchild (1970) and Fisher (1980) which cover the ground surrounding the base of a corn plant capturing insects that emerge while allowing the corn plant to grow. Insect collection cups on emergence cages were changed weekly for seven weeks, ending mid August (26 June-5 July; 6 July-12 July; 13 July-19 July; 20 July-26 July; 27 July-2 Aug.; 3 Aug.-9 Aug.; 10 Aug.-16 Aug.). Soybean fields and continuous corn fields were monitored with six Pherocon AM sticky traps that were changed weekly for three weeks (27 July-2 Aug.; 3 Aug.-9 Aug.; 10 Aug.-16 Aug.).

**Diabrotica spp. Abundance.** Two years of Pherocon AM sticky trap data from corn fields presented the opportunity to observe regional level abundance of two similar corn pests. *Diabrotica barberi* and *D. v. virgifera* captured from sticky traps corn fields 2008 and continuous corn fields in 2009 were totaled per field site. For each field site total *D. v. virgifera* abundance was divided by the total abundance of both *D. barberi* and *D. v. virgifera*, providing the proportion of *D. v. virgifera*. Field sites with proportion of *D. v. virgifera* values $\geq 0.7$ were labeled as *D. v. virgifera* dominated, while field sites with proportion of *D. v. virgifera* values of $\leq 0.3$ were labeled as *D. barberi* dominated. Proportion of *D. v. virgifera* values between 0.69 and 0.31 were labeled as neutral field sites.
Analysis. ArcGIS 9.3 (ESRI, Redlands, CA) geostatistical analyst was used to create predicted surfaces by Inverse Distance Weighting (IDW) interpolation for the eastern half of Iowa. Predicted surfaces determine variable values for areas that were not actually sampled using data from georeferenced sampled field sites. IDW interpolation is deterministic and calculates values for unsampled areas using the surrounding sampled field sites with closer field sites having greater influence than those further away. These interpolations are also exact, so predicted surface values will equal the actual value of a georeferenced point at that point’s respective location. The power value control or exponent used for these IDW’s was 2, and the IDW surface was estimated by the nearest 10-15 georeferenced sampled field sites.

Abundance of *D. barberi* and *D. v. virgifera* in 2008 corn fields and 2009 continuous corn fields were pooled by species to create IDW surfaces estimating abundance of each species in corn fields over eastern Iowa. Totals of *D. barberi* and *D. v. virgifera* from corn fields sampled in 2008 were counted per sticky trap. Totals from each species per sticky trap were then averaged by the number of days the sticky trap was exposed to the field to produce average *D. barberi* or *D. v. virgifera* captured per sticky trap per day (average capture). Data from continuous corn fields sampled in 2009 were calculated using the same technique. Six of 17 field sites collected data from multiple continuous corn fields sampled in 2009, and at those 6 field sites data from both continuous corn fields were pooled. IDW interpolations of abundance of *D. barberi* and *D. v. virgifera* in corn fields throughout eastern Iowa were based on average capture of each respective species.

Peak abundance of *D. barberi* and *D. v. virgifera* in 2008 corn fields and 2009 continuous corn fields were pooled by species to create IDW surfaces estimating peak abundance of each species in corn fields throughout eastern Iowa. Sticky traps were changed every week for 3 weeks in both years sampled. Weekly average *D. barberi* and *D. v. virgifera* captured per sticky trap per day were calculated for each week at each field site from 2008 corn field and 2009 continuous corn field data. The peak weekly average capture per sticky trap per day (peak weekly capture) was the highest value
of the three weeks sampled. IDW interpolations of peak abundance of *D. barberi* and *D. v. virgifera* in corn fields throughout eastern Iowa was based on the peak weekly capture of each species.

Combined abundance of *D. barberi* and *D. v. virgifera* in 2008 corn fields and 2009 continuous corn fields were used to create an IDW surface estimating average capture of both species in corn fields throughout eastern Iowa. Combined average capture was calculated in a manner similar to the calculation of average capture of *D. barberi* or *D. v. virgifera* except that the total of both species per sticky trap was averaged by the number of days the sticky trap was exposed to the field. The IDW interpolation of combined abundance in corn fields was based on combined average capture data. Combined peak abundance of *D. barberi* and *D. v. virgifera* in 2008 corn fields and 2009 continuous corn fields was also used to create IDW interpolations that were based on similarly calculated combined peak weekly averages of *D. barberi* and *D. v. virgifera* in corn fields.

Abundance of *D. v. virgifera* in soybean fields in 2008 and 2009 was pooled to create an IDW surface estimating abundance of *D. v. virgifera* in soybean fields throughout eastern Iowa. Average capture of *D. v. virgifera* in soybean fields was calculated similarly to average capture in corn fields. IDW interpolation of abundance of *D. v. virgifera* in soybean fields throughout eastern Iowa was based on average capture of *D. v. virgifera* in soybean fields. IDW interpolation of peak abundance of *D. v. virgifera* in soybean fields was also created using peak weekly capture of *D. v. virgifera* in soybean fields.

Emergence from first-year corn by *D. barberi* in 2009 was used to create an IDW surface estimating total capture of *D. barberi* per emergence cage throughout eastern Iowa. Emerging *D. barberi* from the 7 week sampling period were totaled per emergence cage and divided by the number of cages in each first-year corn field. Average total capture of *D. barberi* per emergence cage in first-year corn fields was calculated per field site. The IDW interpolation of average *D. barberi* per emergence cage throughout eastern Iowa was based on average total capture of *D. barberi* per emergence cage in first-year corn fields. A second predictive surface was created for maximum total
capture by an emergence cage which was estimated using the highest total capture by an emergence cage occurring at each field site. As the number of georeferenced sampled field sites was only 17 in 2009, these IDW’s were estimated by the nearest 10-15 georeferenced sampled field sites. IDW interpolations of emergence from first-year corn by *D. v. virgifera* in 2009 were not created as *D. v. virgifera* emergence was very low.

Peak weekly capture of *D. v. virgifera* in soybean fields was again used to estimate peak weekly abundance of *D. v. virgifera* throughout eastern Iowan soybean fields, this time adjusting for peak weekly abundance of *D. v. virgifera* in corn fields. The regression of log transformed peak weekly capture of *D. v. virgifera* in soybean fields and log transformed peak weekly capture of *D. v. virgifera* in corn fields was used to create adjusted peak weekly capture of *D. v. virgifera* in soybean fields. This adjusted peak weekly capture produced a more conservative IDW interpolation of peak weekly capture of *D. v. virgifera* in soybean fields throughout eastern Iowa.

Proportion of *D. v. virgifera* in 2008 corn fields and 2009 continuous corn fields were pooled to create an IDW surface estimating proportion of *D. v. virgifera* in corn fields throughout eastern Iowa. Proportion of *D. v. virgifera* was measured by totaling capture of both species from sticky cards and then dividing capture of *D. v. virgifera* by total capture of both species per field site. Data categories used in by the IDW interpolation included *D. v. virgifera* dominated fields, *D. barberi* dominated fields, and neutral fields values as described above.

Correlation and regression analyses were used to measure relationships between average capture in corn and soybean fields for both species using PROC CORR and PROC REG respectively in SAS 9.2 (SAS Institute Inc. 2008). Peak weekly capture for both species in corn and soybean fields, adjusted peak weekly capture for *D. v. virgifera* in corn and soybean fields, and average and peak weekly capture in corn fields of *D. barberi* and *D. v. virgifera* were also analyzed using PROC CORR and PROC REG in SAS 9.2 (SAS Institute Inc. 2008).
G-tests of independence were used comparing frequency of neutral fields to dominated fields. Independence was tested using three differed groupings of data; 1) frequency of field sites that are dominated by either species should equal the frequency of neutral field sites, 2) equal proportions of observation found in the three categories used by the proportion of D. v. virgifera IDW interpolation, and 3) frequency of D. v. virgifera dominated field sites, of D. barberi dominate field sites, and of neutral field sites are equal.

**Results**

IDW interpolation using average capture of D. v. virgifera in corn fields predict higher abundance in corn fields in northeast Iowa (Fig. 2A). Dark red contours predicting areas where average capture would be > 1 occur mainly in the northeast state-border counties. As IDW methods are exact at georeferenced data points, two small dark red contours appear around fields that recorded average captures >1 (N1 and C4). Dark blue contours representing estimated average capture of D. v. virgifera <0.25 cover the majority of eastern Iowa. Light red contours predicting average capture between 0.51 and 1 and light blue contours predicting average capture between 0.25 and 0.5 mainly occur in thin bands surrounding the more extreme estimates. White contours represent areas predicting average capture >6, or areas above the economic threshold (Hein and Tollefson 1985), which occurs only in the northeast.

The predictive surface using peak weekly capture of D. v. virgifera in corn fields produces similar estimates as average capture of D. v. virgifera (Fig. 2B). Peak weekly capture contour values and colors are exact to average capture contour values and colors, and these values remained consistent with all interpolations of D. barberi and D. v. virgifera abundance in corn fields. The white contour is found in the northeast of the interpolated area and surrounds field site N10, which recorded > 16 D. v. virgifera /sticky card/ day. Estimates using peak weekly abundance expand the range of the red contour further into the state. Dark blue contours predicting lowest abundance still dominated the south, but become less so using peak weekly capture data. Size of light red contours
were reduced in the east but expanded in the center of the sampling area, while light blue contours expanded and covered many of the northern central counties.

Average capture interpolation of *D. barberi* in corn fields predicted higher abundance in northeast Iowa (Fig. 3A). Abundance was patchy with small, dark red contours occurring in 4 small patches in the center of eastern Iowa. Lowest abundance estimates occurred mainly in the southwest of the interpolated area but other patches were found throughout the sampling area. The IDW interpolation using peak weekly capture *D. barberi* in corn fields expanded the light red contour regions drastically, covering most of the sampling area (Fig. 3B). Areas predicted to have lower average abundance, dark and light blue contours, were reduced while areas of greater abundance, light and dark red contours, expanded.

Interpolations using combined average capture predict larger population of *Diabrotica* spp. to occur in corn field located in the north central and northeast (Fig. 4A). The majority of predicted low abundance, light blue and dark blue contours, were in south central Iowa. Combined populations estimated to be above the economic threshold were found in the same area as predicted in average capture of *D. v. virgifera*, both occurring in the northeast. Combined peak weekly capture IDW interpolations expanded the dark red and white contours, with dark red combined peak weekly captures >1 predicted across the north of the sampling area (Fig. 4B). Lower abundance predictions were almost exclusively found in the southern three tiers of counties.

Abundance of *D. v. virgifera* in soybean fields is almost ubiquitously predicted to be very low (Fig. 5A). The vast majority of the interpolated area is covered by the dark blue contour, predicting average capture <0.1. IDW interpolation indicates that only a small patch in the northeast will record greater average capture, with only one small pocket of the sampling area averaging capture >0.3 *D. v. virgifera* (dark red contours). The interpolation using peak weekly capture increased the size of the areas with higher predicted values, but the majority of the estimated surface is still predicted to have >0.1 peak weekly capture (Fig. 5B). All field sites monitored in both years
did not record populations above the economic threshold of 4.7 *D. v. virgifera*/sticky trap/day in soybean fields (O’Neal et al. 2001), nor did average capture and peak weekly capture IDW predict any such populations (Fig. 5).

Using average total capture of *D. barberi* per emergence cage in first-year corn fields resulted in estimates of greater emergence of *D. barberi* in the center of Iowa (Fig. 6A). Dark red contours, estimated *D. barberi* average total capture >3, were predicted to occur in the north-central and south-central regions of Iowa. Light red contours estimating *D. barberi* average total capture between 2.1 and 3 were found in surrounding dark red contours only. Average total capture between 1 and 2 and <1, light blue and dark blue contours respectively, were predicted throughout the remaining central and east regions of the sampling area. Estimates of maximum total capture of *D. barberi* per emergence cage expanded the range of areas predicted to have greater emergence from first-year corn (Fig. 6B). Higher emergence is predicted to occur in the same areas as average total capture, but now these areas have expanded to cover all northern counties. Lower capture, represented by purple (<1) and dark blue (between 1 and 2.5) contours, are predicted in the southeast. Predicted maximum total emergence appeared to follow a gradient of greater emergence in central and north Iowa which declined towards southeast Iowa. Again, emergence of *D. v. virgifera* was not used to create IDW interpolations as only three adults emerged from first-year corn in 2009.

Average capture and peak weekly capture of *D. v. virgifera* in corn and soybean fields was significantly positively correlated, more so than *D. barberi* (Table 3). Regression analyses revealed evidence of a strong positive relation between *D. v. virgifera* in corn and soybean fields for both average capture and peak weekly capture (Table 4). Log transformation of peak weekly capture also produced a significant relationship ($r^2 = 0.61$; n= 55; P <0.0001) and adjusted peak weekly capture in soybean was calculated based on this regression. IDW interpolation using the adjusted peak weekly capture of *D. v. virgifera* in soybean fields still predicts much of sampling region to be <0.1 (Fig. 7).
Predicted adjusted peak weekly capture is elevated in the northeast boarder counties, similar to peak weekly capture estimates (Fig. 5B) but values within this patch were not as high.

IDW interpolation using the proportion of *D. v. virgifera* in corn fields estimates that dominance of corn fields is spatially segregated (Fig. 8A), though, conversely, results from correlation and regression analyses report positive correlation and a significant relationship (Tables 3 and 4). Predicted corn fields of ≥70% total abundance represented by *D. v. virgifera* (yellow contours) were confined to the east-central Iowa border, while predicted corn fields of ≥70% total abundance represented by *D. barberi* (yellow contours) is estimated to occur throughout south-central and north-central Iowa. Areas interpolated as being neutral were located in a vein running north to south that separated *D. barberi* and *D. v. virgifera* dominated contours as well as in running between the north and south predicted *D. barberi* dominated areas. All three G-tests of independence were significant and rejected the null hypothesis that frequencies of neutral and dominated fields were independent (Table 5).

**Discussion**

Crop rotation has traditionally offered growers an inexpensive and effective option for *D. v. virgifera* and *D. barberi* management. Yields are higher in rotated corn than in corn that is continuously produced (Bullock 1992), however, both of these serious pests of corn have adapted to crop rotation. Rotation-resistant *D. v. virgifera* have adapted to crop rotation through a behavioral loss of ovipositional fidelity (Levine et al. 2002). While rotation-resistant *D. barberi* oviposition still occurs primarily in corn fields, large percentages of eggs express a multiyear extended diapause (Krysan et al. 1984, Levine et al. 1992). Surveys from corn and soybean throughout eastern Iowa illustrate that rotation-resistant *D. v. virgifera* have not yet gained a firm footing in Iowa, rotation-resistant *D. barberi* while present throughout eastern Iowa appear to be of limited economic influence, and that there appears to be limited interaction between the two species.
Overall abundance of *D. v. virgifera* in soybean fields was low throughout 2008 and 2009, and mainly occurred in the northeast portion of the sampling region (Figs. 5A). Onstad et al. (1999) used a minimum of 10 *D. v. virgifera* captured in soybean fields per 100 sweeps from a sweep net to avoid spurious captures in models used to predict the spread of rotation-resistance. Before the adaptation of rotation-resistance the maximum capture of *D. v. virgifera* in soybean fields from 100 sweeps was between 10-16 (Levine 1995). Using regression analysis between captures with sweep nets and captures on sticky traps Onstad et al. (2003a) estimates 10 adults captured per 100 sweeps is equivalent to 1.34 average captured adults/sticky trap/day. Only one field site surveyed recorded peak weekly abundance >1 *D. v. virgifera* captured/ sticky trap/day. This was recorded in 2008 at N10, which stood out in all IDW interpolations as having the largest *D. v. virgifera* populations in soybeans (Figs. 5 and 7).

Adult *D. v. virgifera* prefer to feed on corn pollen, silks, and kernels (Ludwig and Hill 1975), and movement of adults can be effected by the phenological state of a corn crop (Darnell et al. 2000, O’Neal et al. 2002). Historically, as the growing season progresses *D. v. virgifera* abundance become less in continuous corn fields where they emerged and more abundant, especially females, in first-year corn (Godfrey ad Turpin 1983). This may play a role in *D. v. virgifera* rotation-resistance as these females have been shown to be more active and take flight more frequently than rotation-susceptible females (Knolhoff et al. 2006). In a series of surveys of corn and adjacent soybean fields throughout Illinois Spencer et al. (1997, 1998, 1999a) repeatedly found that fields monitored in rotation-resistant affected areas have higher abundance of *D. v. virgifera* in soybean fields than areas where rotation-resistance is absent. Of particular interest are the data from De Kalb County, which then was located just outside the region affected by rotation-resistance. In Spencer et al. (1998) observed minimal capture of *D. v. virgifera* in vial traps placed in soybean fields, but the following year capture in DeKalb County soybean fields increased noticeably (Spencer et al. 1999a).
Similar results are noticeable in data presented here (Figs 2 and 5). There is a clear pattern of elevated *D. v. virgifera* activity in soybean fields in northeast Iowa, nearest to the suspected rotation-resistance invasive front. Interpolations showed larger predicted capture of *D. v. virgifera* in corn fields in northeastern Iowa around field sites N10-12 and C11-14 (Fig. 2). Interpolations of captures in soybean fields from the same area predict greater capture than the rest of sample region (Fig. 5), albeit below the Onstad et al. (1999) model threshold. Interpolations showing larger predicted capture of *D. v. virgifera* in corn fields around field sites N1 and C4 in central Iowa (further away from the suspected rotation-resistance invasive front) (Fig. 2) were predicted to have very little capture of *D. v. virgifera* in soybean fields (Fig. 5). Correlation between *D. v. virgifera* abundance in corn and soybean fields was both significant and positive (Table 3), and regression between *D. v. virgifera* abundance in corn and soybean fields was more tightly linked than *D. barberi* abundance in corn and soybean fields (Table 4). IDW interpolation after correcting for corn field abundance still predicts a small area in northeast Iowa with elevated captures of *D. v. virgifera* in soybean fields (Fig. 7).

Models of rotation-resistant *D. v. virgifera* range expansion have been created using storm and wind direction (Onstad et al. 1999, 2003a). As storms in the Midwest generally move eastward the direction of rotation-resistant range expansion also is predicted, and observed, to be mainly eastwardly. Onstad et al. (1999) predicted that rotation-resistance may reach Iowa (west of the rotation-resistant epicenter) within the next 10 years, roughly estimated as 2009, but that increased landscape diversity may reduce the rate of advancement (Onstad et al. 2003a). Similar models used to estimate the spread of rotation resistance have predicted the behavior to invade eastern Iowa by 2011 (Levine et al. 2002). The small populations in the northeast may be the forefront of the invading rotation-resistant behavior. Observations reported here are not the first of populations of *D. v. virgifera* in Iowa soybean fields since small pockets of *D. v. virgifera* activity were also reported in eastern Iowa soybean fields in 2005 (Prasifka et al. 2006). Invading populations are strongly
influenced by stochastic events (Liebhold and Tobin 2008) and small populations are less likely to establish (MacArthur and Wilson 1967). Small population may also have trouble establishing due to the Allee effect, where population growth decreases with decreasing abundance (Courchamp et al. 1999).

Distribution of extended diapause expands over much of the Midwest (Landis et al. 1992, Levine et al. 1992, Krysan 1993), though expression of rotation-resistance does vary (Steffey et al. 1992). Reported injury to first-year corn by rotation-resistant *D. barberi* in Iowa was primarily located in the northwest, and injured first-year corn represented only a minority of all rotated corn (Foster, 1987).

Frequency of resistance to crop rotation is directly proportional the percentage of annually rotated corn (Krafsur 1995). If rotation-resistant *D. barberi* are exposed to a consistent pattern of crop rotation over long periods of time the risk of economically damaging populations increase (Fisher et al. 1994). Mating between rotation-resistant and rotation-susceptible *D. barberi* is random and unrestricted, which can act to slow the rate of spread of rotation resistance (Krafsur 1995). IDW interpolations of total and maximum capture of *D. barberi* from emergence cages in first-year corn provide evidence of this slow dispersal (Fig. 6). The western transect in 2009 ran almost parallel to the eastward extent of injury to first-year corn reported by Foster (1987). North-central Iowa is predicted to have higher total capture of *D. barberi* from first-year corn and this area is located similarly to areas reporting injury to first-year corn in 1987. Maximum capture interpolation shows that expression of extended diapause expanded possibly three counties eastward and two counties southward in 22 years based on higher levels of emergence (Fig 6B). Expansion of extended diapause range in Iowa appears to have made little eastward and southward progress according to the IDW interpolations.

Rotation-resistant *D. barberi* may be present over much of the Corn Belt but in some areas it is rarely of economic concern (Gray et al. 1998). Foster (1987) did not report any injury to first-year
corn in eastern Iowa but this could be because expression of extended diapause in eastern Iowa is relatively low and produced little if any noticeable injury, which is congruent to IDW interpolations predicting capture of *D. barberi* (Fig. 6). Another hypothesis is that *D. barberi* are not as capable of causing injury as *D. v. virgifera* and therefore some populations go unnoticed. In comparisons between *D. v. virgifera* and *D. barberi* infested plots Fisher (1985) observed that the *D. barberi* caused less damage. Field trials testing efficacy of soil insecticide on corn yield and *D. barberi* found a nonsignificant relationship between density of emerging adults and yield, showing evidence that corn plants may tolerate infestation by *D. barberi* (Cormier and Martel 1997).

Genetically modified crops, such as corn expressing Bt toxins, increase the effectiveness of corn host plant resistance. As adaption of Bt corn increases, interactions between rotation-resistance and Bt corn becomes more important. Models created to simulate Bt corn’s effect on rotation-resistant *D. v. virgifera* showed that planting rotated corn fields with an effective Bt trait could prevent adaptation to crop rotation (Onstad et al. 2003b). When comparable models were created to include simulation of *D. v. virgifera* resistance to both Bt corn and crop rotation, cropping systems with rotated corn planted with a Bt trait and continuous corn planted without a Bt trait was effective at preventing resistance to both Bt and crop rotation (Crowder and Onstad 2005). Rotation-resistance in *D. barberi* is thought to have a more convoluted relationship with Bt corn. Extended diapause both increased susceptibility to Bt corn by reintroducing older, less resistant alleles and decreased susceptibility by increasing population recovery from heavy losses (Mitchell and Onstad 2005).

Displacement in insect communities can be caused by a combination of competitive and noncompetitive factors, and many cases of displacement occur between closely related species (Reitz and Trumble 2002). Both *D. v. virgifera* and *D. barberi* are corn pests with very similar life histories (Chiang 1973). In Hardin (1960) published the competitive exclusion principle; species that occupy the same niche cannot do so indefinitely. Under close enough examination every species will invariably occupy a unique niche, though competitive exclusion still exists (Rockwood, 2006). In a
study of 13 different tropical Coleopteran species that all utilized similar food and habitat, both unlimited, found no evidence of competition (Strong 1982), though significant interactions can occur between herbaceous arthropods in the presence of unlimited food (Hairston et al. 1960). Competing species may also coexist where catastrophic events regularly destroy habitats and new ones are created (Hutchinson 1953). Habitat for *D. v. virgifera* and *D. barberi* is constantly being demolished and recreated as corn fields are rotated to and from corn. IDW interpolations of proportion of *D. v. virgifera* throughout eastern Iowa predicted unbalanced dominance, with fields estimated to be geographically segregated by species (Fig. 8). While abundance was positively correlated between *D. v. virgifera* and *D. barberi* (Table 3) tests of independence showed that there were significantly more fields dominated than if relative frequencies were independent (Table 5). This evidence alone is not proof of competition, which can be difficult to prove in conditions other than the laboratory and simple field tests (Schoener 1983).

Crop rotation is valuable to corn producers and loss of this inexpensive control would negatively impact producers. IDW interpolation based on surveys of eastern Iowa found minimal evidence of rotation-resistant *D. v. virgifera*, though interpolations estimated relatively higher abundance of *D. v. virgifera* in soybeans fields located in the northeast. This may be the western front of rotation-resistant *D. v. virgifera* beginning to establish small populations. IDW interpolation also predicted rotation-resistant *D. barberi* throughout eastern Iowa but were more abundant in the north-central and south-central regions. These infestations may not often be of economic concern.

**Acknowledgments**

We thank Pioneer Hi-Bred International, Monsanto, Bayer Crop Science, and Syngenta for funding this research. Thanks to the many Iowa State University regional and county field agronomists who helped identify our fields. Thanks to Patricia Prasifka and Nicholas Schmidt for help getting off and running. Special thanks to Ben Lundgren and Brandon Sorgatz who in 2009 helped cover half the world in Iowa.
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http://www.epa.gov/oppbppd1/biopesticides/pips/smartstax-factsheet.pdf


Table 1. 2008 Rotation-resistance monitoring field sites in eastern Iowa labeled from west to east

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<thead>
<tr>
<th>Site #</th>
<th>Northern Transect GPS Coordinates</th>
<th>Site #</th>
<th>Central Transect GPS Coordinates</th>
<th>Site #</th>
<th>Southern Transect GPS Coordinates</th>
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<td>C1</td>
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<td>N 41.366°; W 093.731°</td>
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<th>Site #</th>
<th>GPS Coordinates</th>
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<td>E1</td>
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<td></td>
<td>W3</td>
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<td>N 42.668⁰; W 091.800⁰</td>
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Table 3. Results of correlation analyses from average capture and peak weekly capture data

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<th>Model</th>
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<th>r</th>
<th>P</th>
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<tbody>
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<td>Average capture/</td>
<td>D. v. v. Corn = D. v. v. Soybean</td>
<td>53</td>
<td>0.807</td>
<td>&lt;0.0001</td>
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<td>sticky card/day</td>
<td>D. b. Corn = D. b. Soybean</td>
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<td>0.372</td>
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<td>D. v. v. Corn = D. b. Corn</td>
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<td>0.574</td>
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<td>Peak weekly average</td>
<td>D. v. v. Corn = D. v. v. Soybean</td>
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<td>0.891</td>
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Table 4. Linear regression analyses with coefficients of determination from average capture and peak weekly capture data

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<td>Peak weekly average capture/ sticky card/day</td>
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Table 5. Results of G-tests of independence from total abundance in 2008 corn fields and 2009 continuous corn fields data

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<th>G</th>
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<td>4.19</td>
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<td>Dominated Fields : 38 Dominated Fields : 27.5</td>
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<td>#2</td>
<td>Neutral Fields : 7 Neutral Fields : 22</td>
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<td>10.94</td>
<td>&gt;0.0001</td>
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<td>Dominated Fields : 48 Dominated Fields : 33</td>
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</tr>
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<td>#3</td>
<td>Neutral Fields : 6 Neutral Fields : 18.3</td>
<td>1</td>
<td>8.32</td>
<td>0.003</td>
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<tr>
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<td>Dominated Fields : 49 Dominated Fields : 36.6</td>
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</tbody>
</table>

1 Frequency of field sites that are dominated by either species equals the frequency of neutral field sites

2 Equal proportions of observation found in ≥70 *D. v. virgifera* dominated field sites, ≥70 *D. barberi* dominate field sites, and neutral sites

3 Frequency of *D. v. virgifera* dominated field sites, of *D. barberi* dominate field sites, and of neutral field sites are all equal
Fig. 1. Map of 2008 and 2009 field sites. The 3 transects from 2008 run east to west and are highlighted in blue, with field sites represented by blue points. The 2 transects from 2009 run north to south and are highlighted in red, with field sites represented by red points.
Fig. 2. IDW interpolation of *D. v. virgifera* abundance in corn fields using average capture of *D. v. virgifera* from 2008 corn fields and 2009 continuous corn fields (A). IDW interpolation of peak abundance using peak weekly capture of *D. v. virgifera* from 2008 corn fields and 2009 continuous corn fields (B).
Fig. 3. IDW interpolation of *D. barberi* abundance in corn fields using average capture of *D. barberi* from 2008 corn fields and 2009 continuous corn fields (A). IDW interpolation of peak abundance using peak weekly capture of *D. barberi* from 2008 corn fields and 2009 continuous corn fields (B).
Fig. 4. IDW interpolation of combined *D. barberi* and *D. v. virgifera* abundance in corn fields using combined average capture of *D. barberi* and *D. v. virgifera* from 2008 corn fields and 2009 continuous corn fields (A). IDW interpolation of combined peak abundance of *D. barberi* and *D. v. virgifera* abundance in corn fields using combined peak weekly capture of *D. barberi* and *D. v. virgifera* capture from 2008 corn fields and 2009 continuous corn fields (B).
Fig. 5. IDW interpolation of *D. v. virgifera* abundance in soybean fields using average capture of *D. v. virgifera* from 2008 and 2009 soybean fields (A). IDW interpolation of peak abundance using peak weekly capture of *D. v. virgifera* from 2008 and 2009 soybean fields (B).
Fig. 6. IDW interpolation of average emergence of *D. barberi* from first-year corn using average total capture of *D. barberi* per emergence cage based on data from 2009 (A). IDW interpolation of maximum emergence of *D. barberi* from first-year corn using maximum total capture of *D. barberi* per emergence cage based on data from 2009 (B).
Fig. 7. IDW interpolation of abundance of *D. v. virgifera* in soybean fields using peak weekly capture of *D. v. virgifera* from 2008 and 2009 soybean fields adjusted for peak weekly capture of *D. v. virgifera* in 2008 corn fields and 2009 continuous corn fields.
Fig. 8. IDW interpolation of field dominance by species using proportion of *D. v. virgifera* in corn fields.
Chapter. 3 Effect of Soybean Varieties on Western Corn Rootworm

Survival and Fecundity

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Abstract

The western corn rootworm \textit{Diabrotica virgifera virgifera} LeConte (Coleoptera: Chrysomelidae) is a major pest of corn in North America. Corn growers traditionally use crop rotation as an inexpensive and effective tool to prevent yield loss from \textit{Diabrotica} spp. However, in regions of the Midwest, \textit{D. v. virgifera} has evolved resistance to crop rotation by laying eggs in alternate crops such as soybeans. Soybeans with resistance to soybean aphid \textit{Aphis glycines} Matsumura (Hemiptera: Aphididae) were investigated for affects on survival and fecundity of \textit{D. v. virgifera}. Soybean varieties that reduce the fitness of \textit{D. v. virgifera} may help to delay the evolution of rotation resistance. Two experiments were conducted; one with plants grown in the greenhouse and one with plants grown in the field. Soybeans tested included varieties with \textit{Rag1} and \textit{rag1/rag3} resistance to \textit{A. glycines} and a near \textit{rag1} isolate. Female \textit{D. v. virgifera} were exposed to treatments of corn, corn alternating with starvation, or corn and alternating with soybean. Survival, oviposition, and consumption were recorded every two days. In the experiment that used plants grown in the greenhouse, \textit{D. v. virgifera} fed corn continuously had significantly greater egg production than treatments that alternated between corn and the soybean varieties. For both experiments, there were no significant differences between soybean varieties for survival, egg production, or soybean leaf area consumed. Soybean varieties with \textit{Rag1} and \textit{rag1/rag3} resistance to \textit{A. glycines} appear not to impose natural selection for or against rotation resistance in \textit{D. v. virgifera}.

Introduction
Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae), the western corn rootworm, is a major pest of corn in North America. Yield loss from Diabrotica spp. feeding and attempted control measures is estimated to cost greater than $1 billion annually in the United States alone (Metcalf 1986). D. v. virgifera is a univoltine species that primarily consumes corn tissue throughout its life cycle (Chiang 1973). Larvae hatch in spring and feed on corn roots, but roots of other grass species can also sustain development (Oyediran et al. 2004). Root feeding can inhibit moisture and nutrient uptake as well as cause lodging of plants and yield loss (Spike and Tollefson 1991). Lodged plants can reduce yield by complicating mechanical harvest (Riedell 1990). Larvae pupate in the soil and emerge as early as July (Levine and Oloumi-Sadeghi 1991). Eggs are oviposited during summer and fall, traditionally in corn fields, where they overwinter in the soil and hatch the following spring.

Crop rotation has traditionally provided inexpensive and effective cultural control of D. v. virgifera in the Midwest. A two-year rotation of corn and soybeans can increase yields by 5 to 20% compared with corn in continuous production (Bullock 1992). Yield advantages gained by two-year rotation of corn and soybeans remain even when increased amounts of fertilizer and pesticide are applied to continuous corn production (Bullock 1992).

The first observed damage to rotated corn by D. v. virgifera occurred in east-central Illinois in 1987 (Gray et al. 1996, Gray et al. 2009). In 1995 the first reported severe damage by D. v. virgifera to annually rotated corn occurred in the same area (Gray et al. 1996). Sixteen first-year corn fields sampled by Gray et al. (1996) averaged damage of 3.72 on the 1-6 Iowa State University root-rating scale (Hills and Peters 1971). Root ratings of 4 indicate one node of root is completely destroyed and that economic loss is possible. The hypothesis that D. v. virgifera females were repelled from corn by pyrethoroids into neighboring soybean fields (Levine and Oloumin-Sadeghi 1996) was quickly dismissed as rotation resistance spread outward from a suspected epicenter (Onstad et al. 1999).

Prolonged egg diapause in D. v. virgifera was also ruled out as a possible cause because only 0.14%
and 0.21% of eggs from problem and non-problem areas, respectively, underwent prolonged diapause (Levine et al. 1992). In a laboratory experiment, Sammons et al. (1997) found that *D. v. virgifera* from known rotation resistant areas were more attracted to soybean than *D. v. virgifera* from areas where rotation resistance was not present. That rotation-resistant *D. v. virgifera* are attracted to soybean was later refuted by free flight wind tunnel experiments (Spencer et al. 1999) and electroantennogram responses of *D. v. virgifera* to both corn and soybean (Hibbard et al. 2002). O’Neal et al. (1999) found a higher percentage of female *D. v. virgifera* captured on Pherocon AM and vile traps in eastern Illinois soybean fields than corn fields. This observation lead to the hypothesis that soybean fields might be suitable oviposition sites for *D. v. virgifera*. O’Neal et al. (2001) found that 27% of the variation in root injury to rotated corn could be explained by *D. v. virgifera* densities in soybean fields the previous year. Rotation-resistance has since spread outward from the epicenter in east-central Illinois, and models using wind and storm patterns have been used to predict the increasing range of rotation-resistance (Onstad et al. 1999, 2003).

Through a behavioral adaption *D. v. virgifera* has circumvented crop rotation by ovipositing eggs in soybean fields (Levine et al. 2002). Feeding on soybean material by *D. v. virgifera* occurs regardless of rotation-resistance (Levine et al. 2002), though rotation-resistant *D. v. virgifera* do not possess a greater capacity to feed on soybeans than rotation-susceptible *D. v. virgifera* (Mabry and Spencer 2003, Mabry et al. 2004). Oviposition by rotation-resistant *D. v. virgifera* is not limited to corn and soybean fields but can also occur in oats, alfalfa, and winter wheat (Rondon and Gray 2004, Schroeder et al. 2005). However, oviposition occurs at higher frequency in soybean fields because soybean is the most common crop besides corn in areas affected by rotation resistance (Onstad et al. 2003). Behavioral based resistance is not unique to *D. v. virgifera*. *Plutella xylostella* L. (Lepidoptera: Plutellidae), the diamondback moth, is a significant pest of family Brassicaceae (Talekar and Shelton, 1993). Sarfraz et al. (2005) found that insecticide-resistant *P. xylostella*
oviposit significantly more eggs around the host plant’s soil-stem interface than the more traditional location of the host leaf, suggesting a behavioral resistance to foliar-applied insecticide.

Host plant resistance can be a valuable pest-management strategy. Traditionally host-plant resistance has been classified into three main categories - nonpreference, antibiosis, and tolerance (Painter 1951). Antibiosis functions as the pest begins to colonize a host plant, causing either direct or indirect negative effects to the pest and this maybe constitutive or induced (Levin 1976). Nonpreference was later changed to antixenosis because antixenosis better defines a plant characteristic as opposed to a pest’s reaction (Kogen and Ortman 1978). Antixenosis refers to a resistance mechanism that deters or reduces pest colonization (Panda and Khush 1995). Lee (1983) describes one case of soybean antixenosis toward *Empoasca fabae* Harris (Homoptera: Cicadelliae) via a dense layer of pubescence preventing the insect from feeding. Tolerance is distinct from antixenosis and antibiosis (Panda and Heinrichs 1983). Tolerance increases a host’s ability to cope with pest colonization and is geared towards the survival of the host independent of the effect of the insect (Panda and Khush 1995).

Host plant resistance in soybeans may affect the fitness of rotation-resistant *D. v. virgifera* and consequently the spread of rotation resistance. In 2000 *Aphis glycines* Matsumura (Hemiptera: Aphididae), the soybean aphid, was found in the Midwest (Hartman et al. 2001). Since this discovery the search for resistance to *A. glycines* in soybeans has been extensive. Hill et al. (2004) found several soybean cultivars that were resistant to *A. glycines* with antibiosis and antixenosis believed to be the main modes of action for resistance, including cultivars Dowling, Jackson, and PI 71506. Li et al. (2004) found that *A. glycines* placed on a Dowling cultivar migrated away from the leaf and postulated that reduced feeding may be caused by antibiosis, which was later found to be controlled by a single dominate gene designated as *Rag1* (Hill et al. 2006a). The designation *Rag1* mean resistance to *Aphis glycines*, the number ‘1’ specifies that this is the first gene identified for resistance in soybeans to *A. glycines*, and the capital ‘R’ signifying that the resistance is dominantly inherited.
Further exploration of PI 71506 found 4 accessions to be resistant, two with antibiosis resistance and two with antixenosis (Mensah et al. 2005). One of these accessions, PI 567598B, has resistance that is controlled by two recessive genes (Mensah et al. 2008). This resistance is thought to be controlled by the recessively inherited \textit{rag1} and \textit{rag3} genes (D. Wang, personal communication).

As the soybean landscape changes to cope with \textit{A. glycines} what, if any, affect will these changes have on \textit{D. v. virgifera} entering soybean fields? The purpose of this study was to evaluate how varieties of soybeans, particularly those varieties with resistance to the soybean pest \textit{A. glycines}, will affect the survival and fecundity of \textit{D. v. virgifera}, and its consumption of soybean leaves. Soybean cultivars that lower the fitness of \textit{D. v. virgifera} could slow the spread of rotation-resistance.

\textbf{Methods}

\textbf{Experiment with Greenhouse Grown Plants.} Corn and soybean used in greenhouse experiments were grown in a greenhouse, maintained at 29\(^\circ\)C and a 16:8 (L:D) h photoperiod, at Iowa State University. Blue River Hybrid (Kelly, Iowa) 36R19 hybrid corn was planted weekly beginning 16 March, 2009 for six weeks. Varieties of soybean were potted starting on 30 March, 2009 then every week for six weeks. Varieties of soybean were chosen based on their resistance to \textit{A. glycines}. One soybean variety was E06905, with antibiosis and antixenosis resistance to \textit{A. glycines} (Chiozza et al. in review). The resistance genes in E06905 are both \textit{rag1} and \textit{rag3} (\textit{rag1/rag3}) (D. Wang 2010, personal communication). The second resistant variety used was LD05-16060, with antibiosis gene \textit{Rag1}, derived from (Dowling x Loda) x SD01-76R(3) (Chiozza et al. in review). The third variety studied was SD01-76R, the near isoline of LD05-16060, missing the resistant \textit{Rag1} gene (designated here as \textit{rag1}) (Chiozza et al. in review).

Three strains of \textit{D. v. virgifera} were used in this experiment. Two strains were captured from the field in late August and early September of 2008. Suspected rotation-resistant beetles were captured in eastern Iowa from soybean fields and named the rotation-resistant strain. Wild-type beetles, those traditionally ovipositioning in corn fields, were captured from corn fields in central
Iowa and named the wild-type strain. Captured adults from both strains were brought back to the laboratory where eggs were collected and placed in cold storage to induce diapause. Eggs for the third strain were supplied by the North Central Agricultural Research Laboratory (NCARL) in Brookings, South Dakota and were reared to adults in the laboratory. The third strain of *D. v. virgifera* was originally selected for early hatch over nine generations (Branson 1976) and has since undergone over 190 generations without out-crossing (Kim et al. 2007). This third strain was named the standard strain. Diapausing eggs from rotation-resistant and wild-type strains were removed from diapause in mid-April 2009 and reared to adults which occurred synchronously with adult emergence of the standard strain.

Emerging adults from each strain were sexed and placed in 473 mL clear polypropylene mating containers (Johnson Paper and Supply Co., Minneapolis, MN) where populations were maintained at a maximum of 50 individuals per container. Sex ratio within containers was maintained at 1 female: 1.2-1.5 males. Male *D. v. virgifera* can mate with multiple females (Branson et al. 1977) and it was desired for females to not be mate limited. Individuals within containers were given freshly diced corn ear and silk and a 1.5% agar solution for moisture. Mating containers were changed every third day and fresh corn tissue and agar was added. After ten days males were discarded. Female *D. v. virgifera* were randomly assigned to one of five diet treatments that alternated between 4 days on treatment diets and 4 days on corn tissue. Diet treatments included soybean with *rag1/rag3*, soybean with *Rag1*, soybean isolate of *rag1*, corn tissue only, and starvation. For treatments with soybean, leaf tissue was cut using a size 12 cork borer from freshly trimmed trifoliolate leaves. When individuals were fed corn tissue, finely cut corn kernels and silks were homogenized before they were fed to adults. Individual females were placed in 10 cm Petri dishes and given their corresponding food source on moistened filter paper along with a small piece of 1.5% agar, and oviposition substrate. The ovipositional substrate was created following Mabry et al. (2004). Ovipositional substrate was created by mixing 6.9% agar solution with finely sieved soil
and pouring this mixture over a textured tray. Four cm Petri dishes were used to cut out disks of the ovipositional substrate. Centers of the disks were removed using a size 12 cork borer to create a textured ring that the females used for oviposition. All treatments were kept in an environmental chamber at 25°C and a 16:8 (L:D) h photoperiod.

Petri dishes and all contents were changed every other day and survival of females recorded. At that time, each dish was carefully inspected for eggs. Soybean leaf tissue was removed and leaf area immediately measured under a microscope using Motic Image Plus 2.0 (Motic China Group Co.). To determine the area eaten, leaf disks were compared against control leaf disks that were not exposed to *D. v. virgifera*, but otherwise treated the same. Area consumed was measured as area from control leaf disk (cm$^2$) – leaf disk fed to a female (cm$^2$).

**Experiment with Field Grown Plants.** Corn and soybeans were grown at Johnson farm, an Iowa State University Research and Demonstration Farm immediately south of Ames, Iowa. Varieties planted for the field grown plant experiment were identical to those grown in the greenhouse. Corn was planted 8 May, 15 May, 5 June, and 11 June and soybean varieties on 28 May, 3 June, and 9 June, 2009. Multiple planting dates were used to ensure a ready supply of corn and soybean material.

Two different strains of *D. v. virgifera* were used for this experiment. The first stain was collected from tents covering a continuous corn crop located at the Johnson farm. Tents were erected over corn and adult beetles were collected daily as they emerged. The other strain was a non-diapausning line from NCARL in Brookings, South Dakota. Though of different genetic background than the standard strain. This non-diapausning strain was the product of a single outcross of standard strain females with males from four different locations throughout the Midwest in 2004 and then selected for short diapause (C. Nielson, personal communication). This non-diapausning strain was designated as the short diapause base population (SDBP).
Creation of mating containers, assignment of females to diet treatments, and data collection were the same as the experiment with greenhouse grown plants.

**Analysis.** Fecundity and survival were compared among strain and diet treatment with analysis of variance (ANOVA) using PROC GLM in SAS 9.2 software (SAS institute Inc. 2008). All analyses were adjusted for multiple comparisons using Dunn-Sidak correction. Analyses were performed separately for the experiment conducted with greenhouse grown plants and the experiment using field grown plants. Comparisons among diet treatments alternating with soybean were similarly preformed using ANOVAs for data on daily and total lifetime soybean consumption for both experiments. These analyses of consumption were also adjusted for multiple comparisons using Dunn-Sidak correction.

Fecundity was measured as total egg production by an individual. Survival was measured as the number of days a female survived after they were removed from containers with males and placed in diet treatments. Daily soybean consumption was calculated by adding soybean area consumed by an individual *D. v. virgifera* and then dividing by the number of days it survived. Total lifetime soybean consumption was the total area consumed by a female.

Sample size per treatment for the experiment with greenhouse grown plants were as follows; corn n=31; starvation n= 32; soybean with *rag1/rag3* n=34; soybean with *Rag1* n =33; near isoline of soybean with *rag1* n=32. Sample size for *D. v. virgifera* strains were wild-type n=69; rotation-resistant n=61; and standard n=32, which were distributed equally among the diet treatments. Sample size from the experiment with field grown plants were n=18 individuals per diet treatment and n=90 individual per *D. v. virgifera* strain.

**Results**

**Experiment with Greenhouse Grown Plants.** Survival differed significantly among diet treatments in the experiment with greenhouse grown plants (Table 1). Individuals assigned to the corn only treatment survived longer than those on starvation treatments, *rag1* isoline soybean, and
soybean with *Rag1* resistance (Fig. 1A). *D. v. virgifera* fed soybeans with *rag1/rag3* resistance did not differ from any of the other 4 treatments. Survival differed significantly among *D. v. virgifera* strains (Table 1), with the wild-type and the rotation-resistant strains surviving almost twice as long as the standard strain (Fig. 1A).

There were significant differences in egg production among diet treatments (Table 1). Individuals that were fed corn continuously produced significantly more eggs than all the other diet treatments (Fig. 2A). The remaining diet treatments did not differ statistically. Egg production by individual did not differ significantly among strains (Table 1). The suspected rotation-resistant strain produced the most eggs per female (Fig. 1B). The wild-type and standard strains oviposited fewer eggs but were not significantly different.

Daily consumption of soybean did not differ significantly between the three soybean varieties (Fig. 3A, Table 1). Total lifetime soybean consumption also did not differ significantly between the three varieties (Fig. 4A, Table 1), with varieties being preferred in the same order as daily consumption. Daily consumption of soybean did differ significantly between strains of *D. v. virgifera* (Fig. 3A, Table 1). The wild-type and suspected rotation-resistant strains both consumed more soybean leaf material than the standard strain. Significant differences were also found when analyzing total lifetime soybean consumed by strain, again the wild-type and suspected rotation-resistant strains consuming more soybean leaf material than the standard strain (Fig. 4A, Table 1).

**Experiment with Field Grown Plants.** There were no significant differences in survival among any of the diet treatments (Table 2), though individuals fed only corn survived the longest while those in the starvation treatment survived the fewest number of days (Fig. 2B). Comparison between the field strain and SDBP strain showed no statistical difference in survival among any of the diet treatments (Fig. 2A, Table 2).

Fecundity differed significantly among diet treatments (Table 2). Individuals fed only corn produced more eggs than individuals in all other diet treatments except in the diet treatment with
Rag1 soybean (Fig. 2B). Adult *D. v. virgifera* that were fed diets that alternated with Rag1 soybeans and corn did not differ significantly in fecundity from any other treatment. No statistical differences in fecundity were found between strains (Table 2). Individuals from the field strain produced fewer total eggs than the SDBP strain (Fig. 2B).

Daily consumption did not significantly differ among diet treatments (Fig. 3B, Table 2). Total lifetime soybean consumption also did not differ significantly among the three soybean diet treatments (Fig. 4B, Table 2). Daily consumption of soybeans did not differ significantly between strains of *D. v. virgifera* in the experiment with field grown plants (Fig. 3B, Table 2), though the field strain consumed more soybean material per day than the SDBP strain. However, total lifetime consumption of soybean leaf tissue did differ significantly between strains, with the field strain consuming more than the SDBP strain (Fig. 4B, Table 2).

**Discussion**

Corn-soybean crop rotation is advantageous because it increases yield and controls *Diabrotica* spp. Rotation-resistant *D. v. virgifera* appear to have lower fidelity for oviposition in corn and will readily oviposit in soybean fields (Pierce and Gray 2006), which are more frequently available to rotation-resistant *D. v. virgifera* than other non-corn agriculture crops (Onstad et al. 2003). The introduction of *A. glycines* to soybean production in the United States has increased the need to develop lines with host-plant resistance to *A. glycines*. Yield loss in soybeans from *A. glycines* has been documented to exceed 50% (Wang et al. 1996, Ostlie 2002). Changes in the soybean varieties that are planted may affect rotation-resistant *D. v. virgifera* because this variant feeds on soybeans. We found that soybeans with *A. glycines* resistance genes *Rag1* and *rag1/rag3* did not affect survival, fecundity, or feeding of the *D. v. virgifera*, which suggests that these soybean traits will not impose natural selection for or against rotation-resistance in *D. v. virgifera*.

Observations of damage to first-year corn by *D. v. virgifera* began in the late 1980’s (Gray et al. 1996), though rotation-resistance by oviposition in alternate crops was originally thought unlikely...
(Krysan 1993). This conclusion is understandable as research showed that adult *D. v. virgifera* tend to remain within corn fields (Hill and Mayo 1980). Extended diapause, the physiological adaption to circumvent crop rotation exemplified by *Diabrotica barberi* Smith and Lawrence (Coleoptera: Chrysomelidae) (Chiang 1965, Krysan et al. 1984), was ruled out as the mechanism of rotation-resistance in *D. v. virgifera* as small percentages of *D. v. virgifera* eggs expressed a prolonged diapause (Levine et al. 1992, Levine and Oloumi-Sadeghi 1996). Field studies observing *D. v. virgifera* movement and oviposition would later demonstrate that rotation-resistant *D. v. virgifera* lack oviposition preference (Rondon and Gray 2003, 2004, Schroeder et al. 2005).

Many models have been created predicting the spread of rotation-resistant *D. v. virgifera*. Onstad et al. (1999) predicted that rotation-resistant populations would spread at roughly 10-30km/yr, and that this would be dependant upon prevailing wind and storm direction. After accounting for field observations newer models predicted that range expansion of rotation-resistant *D. v. virgifera* would be slowed by landscape heterogeneity, including non-rotated soybean fields (Onstad et al. 2003). As rotation-resistant *D. v. virgifera* interact more frequently with soybeans understanding changes in soybean agriculture becomes of increasing importance. Factors that have the potential for large scale impact on soybeans, such as the introduction of *A. glycines* and consequential development of soybean lines with host plant resistance, may have potential as a managing tool for rotation-resistant *D. v. virgifera*.

Over 55% of females in soybean fields have been found with soybean in their gut content (Levine et al. 2002). Previous studies have found that there is no attraction to soybean plants in *D. v. virgifera* regardless of rotation-resistance (Spencer et al. 1999, Hibbard et al. 2002) and that wild-type and rotation-resistant populations do not differ in their consumption (O’Neal et al. 2002). Results presented here indicate that *D. v. virgifera*’s lack of preference for soybean continues into varieties with *Rag1* and *rag1/rag3* resistance. Consumption of soybean never differed significantly between the rotation-resistant strain and the wild-type strain, in agreement with results from O’Neal et al.
Consumption of soybean leaf tissue never differed significantly between any of the soybean varieties used (Figs. 3 and 4; Tables 1 and 2). Mabry et al. (2004) argues that the adaptation of rotation resistance in *D. v. virgifera* does not include increased ability to utilize soybean tissue. Evidence from the experiment with greenhouse grown plants, where suspected rotation-resistant individuals were compared to traditional wild-type, supports this argument (Table 1). Individual adults from each strain survived on average 17 days (Fig 1A). Egg production in the experiment with greenhouse plants was also not significantly different between the two strains (Fig.2A). Fecundity from both strains was much lower than *D. v. virgifera*'s reported range of egg production from Boetel and Fuller (1997) where mean total fecundity ranged from 357 to 794 eggs per female. This may have been due to the quality of plants grown in the greenhouse.

It is probable that soybeans are so poor a diet that all present varieties are functionally identical to *D. v. virgifera*. *Rag1* and *rag1/rag3* both convey resistance that targets phloem feeders, while *D. v. virgifera* adults function as defoliators. HC95-15MB and HC95-24MB are two soybean lines with antibiosis resistance to defoliation feeders such as *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae). *Diabrotica virgifera virgifera* captured in sweep nets found no statistical differences among the two defoliation resistant soybeans and a susceptible line (Hammond et al. 2001). This says little about effects these resistant lines may have on *D. v. virgifera* biology but may be a good starting point for future rotation-resistant *D. v. virgifera* management studies.

*Rag1* and *rag1/rag3* are not the only forms of *A. glycines* resistance. *Rag* resistance in cultivar Jackson (Hill et al. 2006b) is similar to *Rag1* resistance in cultivar Dowling (Hill et al. 2006a) as they are both controlled by a single dominate gene and have been mapped to the same position on soybean linkage group M (Li et al. 2007). *Rag2* is a second antibiosis resistance gene mapped in PI 243540 (Mian et al. 2008) and was also determined to be controlled by a single dominate gene (Kang et al. 2008). A second source of *Rag2* may lie in PI 200538 (Hill et al. 2009, Kim et al 2010). PI 567541B is another accession that was found by Mensah et al. (2005) and like PI 567598B
its resistance is controlled by two recessive genes (Mensah et al. 2008). The recessive genes were mapped and designated as \textit{rag1\_provisional} and \textit{rag4} (Zhang et al. 2009). \textit{Rag2} and PI 567541B resistance is especially promising given that different \textit{A. glycines} biotypes appear susceptible that have since overcome resistance found in Dowling and Jackson cultivars (Kim et al. 2008). \textit{Rag1} and \textit{rag1\_rag3} resistance may have had no effect on \textit{D. v. virgifera} biology, but the possibility that other forms of soybean resistance remains largely untested.

It has been well documented that oviposition can occur on alternating corn soybean diets in laboratory experiments (Mabry and Spencer 2003, Mabry et al. 2004) and that adult beetles can survive and produce viable eggs on diets of suboptimal hosts such as squash or sunflower (Siegfried and Mullin 1990). One possible management strategy for rotation-resistance would be to reduce \textit{D. v. virgifera} oviposition in soybean fields, but results here indicate that soybean varieties with \textit{Rag1} and \textit{rag1\_rag3} resistance to \textit{A. glycines} convey no such benefit (Fig. 2). All soybean varieties were not statistically different in egg production from each other or starvation treatments.

Mabry and Spencer (2003) found that adult \textit{D. v. virgifera} could recover from 2 days of no choice soybean feeding and return to full vigor in laboratory studies. Individual adults in this study were given soybean for 4 days to ensure some soybean consumption. Exposure and consumption of soybean had no effect on adult survival among soybean treatments in either experiment. In the experiment with field grown plants there were no differences among diet treatments of any kind, including the treatment where individuals were fed only corn (Fig. 1).

The standard strain used in the experiment with greenhouse grown plants performed poorly when compared to the field captured strains. Total lifetime soybean leaf area consumed was significantly less for laboratory-reared non-diapausing strains in both experiments (Table 1 and 2). Non-diapausing survival and egg production, while not always significantly different, were lower in value than any of the field strains in both experiments (Figs. 1 and 2), with the only exception oviposition in the experiment with field grown plants. Evidence here argues that multiple generation
laboratory-reared strains may have lost some ability to cope with periodic feeding of soybean tissue. The standard colony used in the greenhouse experiment was started with eggs supplied by NCARL’s original non-diapausing strain. Recently this standard strain was tested against other *D. v. virgifera* field and laboratory strains comparing genetic diversity (Kim et al. 2007). The standard strain showed a 15-39% loss in genetic diversity and was viewed as somewhat different from wild populations. This loss of genetic diversity is not unique to *D. v. virgifera* laboratory strains. Stains of laboratory reared *Drosophila melanogaster* (Diptera: Drosophilidae) were shown to decline in genetic diversity over time (Briscoe et al. 1992). When rotation-resistant and wild-type strains were compared to the standard strain, the field strains survived longer and consumed more soybean tissue then the laboratory reared strain. The differences observed here should be considered carefully as loss of genetic diversity may lead to results that do not reflect wild populations (Arias et al. 2005).

Possible effects of *A. glycines* resistant soybeans on *D. v. virgifera* biology could be important to any future management strategies designed to delay the spread or lessen the severity of rotation-resistance behavior. These data suggests that *A. glycines* resistance through *Rag1* and *rag1/rag3* will not impose natural selection for or against the rotation-resistant trait. Soybeans may be so poor a diet that all present varieties are functionally identical to *D. v. virgifera*. Investigations into other types of resistance in soybeans, such as varieties expressing different *Rag* genes or resistance to other soybean pests, remains an potential option for rotation-resistant *D. v. virgifera* management.

**Acknowledgments**

We thank the Iowa Soybean Association for their funding and support, Patrick Weber for his help planting and maintaining our research fields, and the many others who contributed in the lab and field. Thank you all.

**References Cited**


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http://www.soybeans.umn.edu/crop/insects/aphid/aphid_lications_agningsba.htm


Table 1. Analysis of variance for the experiment with greenhouse grown plants

<table>
<thead>
<tr>
<th>Dependant Variable</th>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>P</th>
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</thead>
<tbody>
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<td>Survival</td>
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<td>6.46</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Diet Treatment</td>
<td>4,147</td>
<td>4.27</td>
<td>0.003</td>
</tr>
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<td>Strain*Diet Treatment</td>
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<td>1.46</td>
<td>0.18</td>
</tr>
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<td>Strain</td>
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<td>1.6</td>
<td>0.21</td>
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<td></td>
<td>Diet Treatment</td>
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<td>5.89</td>
<td>&lt;0.001</td>
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<tr>
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<td>Strain*Diet Treatment</td>
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<td>0.45</td>
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<td>Diet Treatment</td>
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<td>Strain*Diet Treatment</td>
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<td></td>
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Table 2. Analysis of variance for the experiment with field grown plants

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<th>$P$</th>
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<tbody>
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<tr>
<td></td>
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<td>5.53</td>
<td>&lt;0.001</td>
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<td>Strain*Diet Treatment</td>
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<td>1.71</td>
<td>0.15</td>
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<tr>
<td></td>
<td>Diet Treatment</td>
<td>2,102</td>
<td>1.12</td>
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<td>Strain*Diet Treatment</td>
<td>2,102</td>
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Fig. 1  Days survived by individual *D. v. virgifera* after beginning the diet treatment with standard error of the mean. The experiment with greenhouse grown plant results (A) are shown on the left hand graph and the experiment with field grown plants results (B) on the right. Letters above the bars denotes significant differences among diet treatments (P = 0.05).
Fig. 2 Egg production of *D. v. virgifera* by diet treatment with standard error of the mean. The experiment with greenhouse grown plant results (A) are shown on the left hand graph and the experiment with field grown plants results (B) on the right. Letters above the bars denotes significant differences among diet treatments (*P* = 0.05).
Fig. 3 Daily consumption of soybean by individual *D. v. virgifera* by diet treatment with standard error of the mean for the experiment with greenhouse grown plants (A) and the experiment with field grown plants (B). Letters above the bars denotes significant differences among diet treatments (P = 0.05).
Fig. 4 Total lifetime consumption of soybean by individual *D. v. virgifera* by diet treatment with standard error of the mean for the experiment with greenhouse grown plants (A) and the experiment with field grown plants (B). Letters above the bars denotes significant differences among diet treatments (*P* = 0.05).
Chapter 4. General Conclusions

Abundance of *Diabrotica virgifera virgifera* Leconte was predicted to occur at higher levels in corn fields of northeast Iowa. This was generally consistent with the direct observations of field sites from both years. The southern transect was especially devoid of *D. v. virgifera* in corn fields which matches Inverse distance weighting (IDW) interpolations of abundance and peak abundance in southern Iowa. Differences between interpolations of abundance and peak abundance were minimal, as the general trend of *D. v. virgifera* occurring at greater abundance in north and less so in the south were evident in both. Abundance of *Diabrotica barberi* Smith and Lawrence in corn fields was predicted to be patchy and greater abundance to occur in the north of the sampling area. Differences between interpolations of abundance and peak abundance were greater for *D. barberi* than *D. v. virgifera*, as peak abundance estimated that areas of higher abundance of *D. barberi* would occur over the majority of the sampling region.

Combined abundance of both species in corn fields was estimated as higher in the north than in the south and interpolations were similar between estimations using average capture and peak weekly capture data. Abundance of *D. barberi* and *D. v. virgifera* in corn fields was positively correlated though interpolations of proportion of *D. v. virgifera* predicted spatial segregation between fields dominated by *D. barberi* and fields dominated by *D. v. virgifera*. It is not clear what, if any, effects each species may have on the other in the field.

Abundance of *D. v. virgifera* in soybean fields was estimated to be higher along the northeast Iowa border. While *D. v. virgifera* presence in soybean fields is not complete evidence of rotation resistance, it is atypical behavior of rotation-susceptible populations. The elevated abundance was only relatively high, as estimates of abundance in soybeans were all very low. Capture of *D. v. virgifera* in soybean fields may be a function of abundance of *D. v. virgifera* in corn fields, but even when we adjusted for abundance in corn fields there were still greater capture of *D. v. virgifera* occurring in northeast soybean fields. The fact that all the relatively higher abundance is predicted to
occur in the same area, and that this area is also near the predicted front of rotation resistance cannot be ignored. What may be occurring is small populations of rotation-resistant *D. v. virgifera* are invading Iowa in advance of the main front and having difficulty establishing due to different biotic and abiotic effects.

Rotation-resistance *D. barberi* were observed emerging more frequently along the western transect, and interpolations of total and maximum emergence of *D. barberi* were consistent with these observations. Range of extended diapause *D. barberi* is expected to increase slowly. Historical distribution of extended diapause in Iowa is similar to interpolations of average and maximum emergence of *D. barberi* from 2009 first-year corn fields in central-north Iowa. However, interpolations show greater emergence occurring in south-central and northeast Iowa. Extended diapause may have extended its range in Iowa, and interpolations of distribution of this adaptation predicts decreasing emergence as estimates move closer to southeast Iowa. Populations expressing extended diapause may not often cause significant injury to first-year corn in eastern Iowa.

It is important to note that IDW interpolation is a method of estimating values for areas that were not sampled. Not all areas locations within a specific color contour will record those specific values associated with that specific color. As with all things biological, there will always be variation.

Currently soybean agriculture is adjusting to the new pest *Aphis glycines* Matsumura and the search for host plant resistance in soybean is ongoing. Soybean with resistance to *A. glycines* in the form of *Rag1* and *rag1/rag3* had no effect on *D. v. virgifera* survival, fecundity, or consumption of soybean tissue when compared to soybean varieties without resistance to *A. glycines* in laboratory assays. The resistant soybean varieties tested here appeared not to impose natural selection for or against rotation resistance in *D. v. virgifera*.