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Effect of Soybean Varieties on Survival and Fecundity of Western Corn Rootworm

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ABSTRACT The western corn rootworm, Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae), is a major pest of corn (Zea mays L.) in North America and has evolved resistance to crop rotation by ovipositing in alternate crops such as soybeans [Glycine max (L.) Merr.]. Through experiments with plants grown in the greenhouse and the field, we tested whether soybeans with resistance to the soybean aphid, Aphis glycines Matsumura (Hemiptera: Aphididae), affected survival, fecundity, and consumption of soybean for D. v. virgifera. Soybean varieties tested included those types resistant to A. glycines (Rag1 and rag1/rag3) and a susceptible near isoline of the Rag1 variety. Females were provided with a diet of corn tissue for 4 d after which they were fed a diet of tissue from one of three soybean varieties for 4 d, starved for 4 d, or fed corn tissue. When fed greenhouse grown plants, strains differed significantly in survival and consumption, but consumption did not differ by variety of soybean. Diet treatment only affected fecundity; individuals fed corn continuously had greater fecundity than those individuals fed soybeans. In the experiment with plants grown in the field, leaf consumption differed among strains and individuals fed corn continuously had greater fecundity than the other treatments. Soybean varieties with Rag1 and rag1/rag3 resistance to A. glycines did not appear to affect the fitness of D. v. virgifera. Thus, planting of these A. glycines-resistant soybean varieties should not directly affect the spread of rotation-resistant D. v. virgifera.

KEY WORDS Aphis glycines, Diabrotica virgifera virgifera, host plant resistance, Rag genes, rotation resistance

Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae), the western corn rootworm, is a major pest of corn (Zea mays L.) in North America. In the United States, yield loss from D. v. virgifera is estimated to cost more than $1 billion annually (Metcalf 1986). Diabrotica virgifera virgifera is a univoltine species that primarily consumes corn tissue (Chiang 1973). Larvae hatch in spring and feed on corn roots, but roots of other grass species also can sustain development (Oyediran et al. 2004). Feeding on corn roots by larval D. v. virgifera inhibits uptake of water and nutrients, causes plant lodging (which complicates mechanical harvesting), and imposes yield loss (Riedell 1990, Spike and Tollefson 1991). Larvae pupate in the soil and adults can begin emerging in July (Levine and Oloumi-Sadeghi 1991). Oviposition occurs throughout the summer and early fall in cornfields, where eggs overwinter in the soil and hatch the next spring.

Crop rotation can provide inexpensive and effective cultural control of D. v. virgifera in the Midwest. Because D. v. virgifera eggs are deposited in cornfields during the summer, and because larvae cannot survive on soybean roots, rotating fields to soybeans [Glycine max (L.) Merr.] kills larvae in the soil (Branson and Ortman 1970). In addition, because D. v. virgifera typically oviposits in corn, fields planted to corn after soybean are not expected to incur rootworm injury. Furthermore, a 2-yr rotation of corn and soybeans can increase yields by 5–20% compared with corn in continuous production (Bullock 1992). Even when the application of fertilizer is increased in continuous corn, it does not match the yield advantage gained by annual rotation of corn and soybean (Bullock 1992).

Because crop rotation kills the progeny of D. v. virgifera females that lay eggs in cornfields, it imposes selection for resistance. Through a behavioral adaptation D. v. virgifera has circumvented crop rotation by ovipositing eggs in soybean fields (Levine et al. 2002). The first observed injury to rotated corn by D. v. virgifera occurred in east-central Illinois in 1987 (Levine et al. 1996, Gray et al. 2009). By 1995 severe injury by D. v. virgifera to annually rotated corn was occurring in the same area (Gray et al. 1996). Eggs of D. v. virgifera recovered from soybean fields (Levine and Oloumi-Sadeghi 1996) confirmed that soybean fields were suitable oviposition sites for D. v. virgifera. Rotation resistance since has spread outward from the epicenter in east-central Illinois and simulation models using wind and storm patterns have been used to predict the future distribution of rotation resistance (Onstad et al. 1999, 2003).
When rotation-resistant *D. v. virgifera* visit soybean fields they eat soybean foliage, although they have no greater ability to use 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 also can occur in oats, alfalfa (*Medicago sativa* L.), 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 after corn in areas affected by rotation resistance (Onstad et al. 2003).

In 2000, *Aphis glycines* Matsumura (Hemiptera: Aphididae), the soybean aphid, was found in the Midwest (Hartman et al. 2001) and quickly became a major insect pest of soybeans in North America (Ragdsdale et al. 2007). *Aphis glycines* overwinters on buckthorn, its primary host, then in spring migrates into soybean fields (Ostlie 2002). Once established on soybean, *A. glycines* feeds on the phloem of the plant, which can reduce yields as much as 50% in some soybean fields (Wang et al. 1996). Many soybean producers choose to treat *A. glycines* infested fields with broad-spectrum insecticides (Olson et al. 2008), but host plant resistance traits can be a valuable addition to pest management strategies. Traditionally, host plant resistance has been classified into three main categories: antixenosis, antibiosis, and tolerance (Painter 1951, Kogan and Ortman 1978). Hill et al. (2004) identified several *A. glycines*-resistant soybean cultivars (i.e., ‘Dowling’, ‘Jackson’, and ‘PI 71506’*) with antibiosis and antixenosis as their likely main modes of action for resistance. Li et al. (2004) found that *A. glycines* placed on a Dowling plant migrated away from the leaf and speculated that reduced feeding may be caused by antibiosis, which was later found to be controlled by a single dominant gene designated as *Rag1* (Hill et al. 2006). The designation *Rag* means “resistance to *Aphis glycines*,” the number one specifies that this was the first gene identified for resistance in soybeans to *A. glycines*, and the capital ‘R’ signifying that the resistance is inherited in a dominant manner.

As the soybean landscape changes in response to *A. glycines*, the planting of soybean varieties with resistance to *A. glycines* may affect the fitness of rotation-resistant *D. v. virgifera* entering soybean fields. The purpose of this study was to evaluate how varieties of soybeans with resistance to *A. glycines* affected the survival and fecundity of *D. v. virgifera*. Soybean cultivars that lower the fitness of *D. v. virgifera* could slow the spread of rotation resistance.

**Methods**

**Experiment with Greenhouse-grown Plants.** Corn and soybean were grown in a greenhouse at Iowa State University maintained at 29°C and a photoperiod of 16:8 (L:D) h. Corn (hybrid 36R19, Blue River Hybrid, Kelly, IA) was planted weekly for 6 wk beginning 16 March 2009. Three soybean varieties also were planted weekly for 6 wk starting on 30 March 2009. Varieties of soybean were chosen based on their resistance to *A. glycines*. One resistant soybean variety was E06905, with *rag1* and *rag3* (*rag1*/*rag3*) (D. Wang, personal communication). The second resistant variety was LD05–16060, with *Rag1* (Chiozza et al. 2010). The third variety was SD01–76R, the susceptible near isole of LD05–16060, which lacked the *Rag1* gene.

Three strains of *D. v. virgifera* were used in this experiment. Two strains were captured from the field in late August and early September of 2008. A Rotation-Resistant Strain was captured in eastern Iowa from soybean fields. A Wild-Type Strain was captured from cornfields in central Iowa. Adults from both strains were brought back to the laboratory where eggs were collected and placed in storage at 8°C. The third strain was the United States Department of Agriculture (USDA) nondiapauing *D. v. virgifera* strain. This strain originally was selected for early hatch over nine generations (Branson 1976) and had been reared in the laboratory for 190 generations (Kim et al. 2007). The USDA-ARS North Central Agricultural Research Laboratory (NCARL) in Brookings, SD provided eggs of this strain, which is referred to hereafter as the Standard Strain. Diapausing eggs from the Rotation-Resistant Strain and Wild-Type Strain were removed from storage in mid-April 2009 and hatched 2 wk later. Insects from all three strains were reared to adults on mats of corn seedlings by using methods similar to Jackson (1985). Blue River Hybrid 36R19 corn, the same variety planted in the greenhouse, was used to produce the seeds mats. Emergence of adults occurred synchronously among all three strains.

Emerging adults from each strain were sexed and placed in 47-ml clear polypropylene 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 one female:1.2–1.5 males. Male *D. v. virgifera* can mate with multiple females (Branson et al. 1977) so females in containers should not have been mated limited. Individuals within containers were given 1.5% agar solid for moisture and fresh corn ear and silk puncture. After 10 d males were discarded.

Female *D. v. virgifera* were placed singly into petri dishes (10 cm in diameter) and randomly assigned to one of five diet treatments. All diet treatments included 4 d of corn feeding alternated with 4 d of feeding on 1) soybean with *rag1*/*rag3*, 2) soybean with *Rag1*, 3) susceptible soybean, 4) additional corn tissue (continuous corn diet), or 5) 4 d of starvation. For treatments with soybean, leaf disks were cut from fresh leaves by using a size 12 cork borer. When individuals were fed corn tissue, finely cut corn kernels and silks were mixed together before they were fed to adults. Food was placed on a disk of moistened filter paper (42.5 mm in diameter) (Whatman, #1 Qualitative, Maidstone, Kent, United Kingdom). In addition, each petri dish contained an oviposition substrate and a small piece of 1.5% agar as a source of water. The ovipositional substrate was made following
Mabry et al. (2004) and was created by mixing 6.9% agar solution with finely sieved soil and pouring this mixture over a textured tray, where it cooled and formed a solid. Disks of the ovipositional substrate were cut using a petri dish (4 cm in diameter). 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 with a photoperiod of 16:8 (L:D) h.

Every other day, the contents of the petri dishes were changed and survival of females recorded. At that time, each dish was inspected carefully and all eggs present were counted and removed. In addition, soybean leaf tissue was removed and leaf area immediately measured under a microscope using Motic Image Plus 2.0 (Motic China Group Co., Ltd, Xiamen, China). 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 calculated as the difference between the area (cm²) of a control disk and the remaining area of each test disk.

**Experiment With Plants Grown in the Field.** Corn and soybeans were grown at an Iowa State University Research and Demonstration Farm immediately south of Ames, IA (Johnson Farm). Varieties and hybrids planted were identical to those plants 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 sufficient corn and soybean material of a consistent phenological state. Corn tissue was collected between R1 and R3 and soybean tissue between R4 and R6.

As the experiment with plants grown in the field occurred immediately after the experiment with greenhouse grown plants, there was insufficient time to rear another generation of the Wild-Type, Rotation-Resistant, and Standard Strains. Instead, two other strains of *D. v. virgifera* were used for the experiment with plants grown in the field. One strain was a Field Stain that was collected from tents covering a continuous corn crop located at the Johnson Farm. Tents were placed over trimmed corn plants and emerging adults were collected daily. The second strain was a short diapause base population (SDBP) Strain obtained from NCARL in Brookings, SD. The SDBP Strain was produced by crossing females of NCARL nondiapausing strain with males from four locations throughout the Midwest in 2004 (C. Nielson, personal communication). Creation of mating containers, assignment of females to diet treatments, and data collection were the same as the experiment with greenhouse grown plants.

**Data Analysis.** Fecundity and survival were compared among strains and diet treatments with two-way analysis of variance by using PROC GLM in SAS 9.2 software (SAS Institute 2008). Pairwise comparisons were made using the PDIf fare option in PROC GLM and alpha values were adjusted for multiple comparisons using a Dunn-Sidak correction. Analyses were performed separately for the experiment conducted with greenhouse grown plants and the experiment using field grown plants. Data on daily and total soybean consumption of soybean leaf tissue were analyzed using two-way analysis of variance that included insect strains and soybean variety as factors. Multiple comparisons were made using the PDIfare options with a Dunn–Sidak correction.

Both experiments contained the factor of insect strain and the factor of soybean variety. These two factors were fully crossed in the experiment with greenhouse grown plants and the experiment with field grown plants. Sample sizes per treatment for the experiment with field grown plants were as follows: corn *N* = 31, starvation *N* = 32, rag1/rag3 soybean *N* = 34, Rag1 soybean *N* = 33, susceptible soybean rag1 *N* = 32. Sample sizes for *D. v. virgifera* strains were Wild-Type Strain *N* = 69, Rotation Resistant Strain *N* = 61, and Standard Strain *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. Within each experiment, all replicates were set up within 2 wk.

**Results**

**Experiment With Greenhouse-Grown Plants.** Survival differed significantly among diet treatments (Table 1). Individuals assigned to the corn treatment survived longer than starvation, susceptible soybean, and Rag1 treatments (Fig. 1A). *Diabrotica virgifera virgifera* fed soybeans with *rag1/rag3* did not differ from any of the treatments. Survival differed significantly among *D. v. virgifera* strains (Table 1). The Wild-Type Strain and Rotation-Resistant Strain survived longer than the Standard Strain (*P* = 0.004 in both cases), but did not differ from one another (*P* = 0.9) (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 the other diet treatments (Fig. 2A). The remaining treatments did not differ statistically. Egg production did not differ significantly among strains (Table 1).

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**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>
<tr>
<td>Survival</td>
<td>Strain</td>
<td>2,147</td>
<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>
<tr>
<td></td>
<td>Strain/diet</td>
<td>8,147</td>
<td>1.46</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecundity</td>
<td>Strain</td>
<td>2,147</td>
<td>1.6</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Diet treatment</td>
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<td>5.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Strain/diet</td>
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<td>0.98</td>
<td>0.45</td>
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<tr>
<td></td>
<td>treatment</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Daily soybean</td>
<td>Strain</td>
<td>2,98</td>
<td>6.24</td>
<td>0.003</td>
</tr>
<tr>
<td>consumption (cm²)</td>
<td>Diet treatment</td>
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<td>1.37</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Strain/diet</td>
<td>4,98</td>
<td>1.08</td>
<td>0.57</td>
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<td></td>
<td>treatment</td>
<td></td>
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<tr>
<td>Total soybean</td>
<td>Strain</td>
<td>2,98</td>
<td>5.78</td>
<td>0.004</td>
</tr>
<tr>
<td>consumption (cm²)</td>
<td>Diet treatment</td>
<td>2,98</td>
<td>1.97</td>
<td>0.15</td>
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<tr>
<td></td>
<td>Strain/diet</td>
<td>4,98</td>
<td>1.15</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
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</table>
Neither daily consumption of soybean leaf tissue nor total life-time consumption differed significantly among soybean variety (Fig. 3A, 4A; Table 1). Daily consumption of soybean did differ significantly among strains of *D. v. virgifera* (Fig. 3A; Table 1), with the Wild-Type Strain and Rotation-Resistant Strain consuming significantly more than the Standard Strain (*P* < 0.05 in both cases) but not differing from one another (*P* = 0.7). Significant differences were also found when analyzing total lifetime soybean consumed by strain (Fig. 4A; Table 1). Again, the Wild-Type Strain and Rotation-Resistant Strain consumed more soybean leaf material than the Standard Strain (*P* < 0.05 in both cases) but did not differ from each other (*P* = 0.9).

**Experiment with Plants Grown in the Field.** There were no significant differences in survival among any of the diet treatments or strains (Table 2), although individuals fed only corn survived the longest while those in the starvation treatment survived the fewest number of days (Fig. 2A).

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* fed *Rag1* soybeans did not differ significantly in fecundity from any other treatment.

Daily consumption of leaf tissue and lifetime consumption of leaf tissue did not significantly differ among soybean varieties (Fig. 3B, 4B; Table 2). Daily consumption of soybeans did not differ significantly between strains of *D. v. virgifera* (Fig. 3B; Table 2). 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**

Annual crop rotation is advantageous because it increases corn yield and helps to manage pest *Diabrotica* spp. However, rotation-resistant *D. v. virgifera* threatens the continued utility of crop rotation. The rotation-resistant variant of *D. v. virgifera* has lower ovipositional fidelity for cornfields and readily will oviposit in soybean fields (Pierce and Gray 2006). The introduction of *A. glycines* to the United States has resulted in yield reductions of up to 50% in soybean fields (Wang et al. 1996, Ostlie 2002), losses of this magnitude have spurred the development of soybean
plants with host plant resistance to *A. glycines*. These *A. glycines*-resistant lines have the potential to affect rotation-resistant *D. v. virgifera* because this variant feeds on soybeans. We found that soybeans with resistance to *A. glycines* based on genes *Rag1* and *rag1/rag3* did not affect survival, fecundity, or leaf consumption of *D. v. virgifera*, indicating that these soybean traits likely will not impose selection for or against rotation-resistance in *D. v. virgifera*.

Onstad et al. (1999) used computer models to predict the spread of the rotation-resistant variant and suggested that their range could expand by as much as 10–30 km per year, depending on prevailing wind and storm direction. Resistance has not spread as quickly as originally predicted (Onstad et al. 2003). Later models predicted that increased landscape heterogeneity, particularly in the percentage of noncorn and nonrotated soybean vegetation, would decrease the rate of expansion for rotation-resistant *D. v. virgifera* (Onstad et al. 2003).

Although we did not find an effect of *A. glycines*-resistant soybeans on *D. v. virgifera*, the presence of economically injurious populations of *A. glycines* may still affect the spread of rotation-resistant *D. v. virgifera*. Broad-spectrum insecticides are the most commonly applied treatment for control of *A. glycines* (Olson et al. 2008), and these insecticides negatively affect a wide range of insect taxa (Ohnesorg et al. 2009). Application of insecticide to soybean fields likely will reduce selection for rotation-resistance in *D. v. virgifera*. The extent to which resistant soybeans affect the rate of *A. glycines*-targeted insecticide application may in turn have an indirect effect of the spread of rotation-resistant *D. v. virgifera*.

There are other forms of soybean resistance to *A. glycines* that might affect the fitness of *D. v. virgifera*. These include *Rag2* and *rag4* (Mensah et al. 2008, Zhang et al. 2009). In addition, soybean varieties with resistance to defoliators may affect the fitness of *D. v. virgifera*, although no such effects have been observed (Hammond et al. 2001).

Compared with insects fed corn continuously, feeding on soybean foliage led to lower survival in the...
experiment with greenhouse grown plants, and lower fecundity in both experiments (Figs. 1 and 2). Mabry and Spencer (2003) found that adult D. v. virgifera could recover from 2 d of feeding on soybean foliage if insects then were fed corn. Rotation-resistant populations have been characterized as being more active than rotation-susceptible populations, which may increase the likelihood of dispersal to and from cornfields (Knollhoff et al. 2006). Soybean consumption alone cannot sustain adult D. v. virgifera and the majority of nutritionally deprived females within a soybean field must return to corn to complete egg development (Mabry et al. 2004), forcing these adults to move between fields. To mimic this pattern of movement, adults in this study were fed soybean for 4 d followed by corn for 4 d.

Rotation-resistant populations are not attracted to soybeans (Spencer et al. 1999, Hibbard et al. 2002), and consumption of soybeans does not differ between rotation-susceptible and rotation-resistant populations (O’Neal et al. 2002). In agreement with these studies, we also did not find a difference in soybean consumption between the Rotation-Resistant Strain and Wild-Type Strain (Figs. 3 and 4; Table 1 and 2). Both the Standard Strain and the SDBP Strain generally displayed lower survival and fecundity when fed soybean than the three field-collected strains, although these effects were not always statistically significant (Figs. 1 and 2). We speculate that this decrease in survival and fecundity may have resulted from the Standard Strain and the SDBP Strain being reared in the laboratory for many generations. Recently, the NCARL nondiapausing strain, the same strain as the Standard Strain used here, was reported to have lost 15–39% of its genetic diversity compared to wild populations of D. v. virgifera (Chiozza, M. V., M. E. O’Neal, and G. C. MacIntosh. 2010). Loss of genetic diversity in large captive populations of Drosophila flies: implications for the genetic management of captive populations. Conserv. Biol. 6: 416–425.

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