

The Effect of Weed Density, Root Senescence, and Egg Density on Western Corn
Rootworm Larval Establishment, Survivorship, and Damage Potential

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

**THE EFFECT OF WEED DENSITY, ROOT SENESCENCE, AND EGG
DENSITY ON WESTERN CORN ROOTWORM LARVAL ESTABLISHMENT,
SURVIVORSHIP, AND DAMAGE POTENTIAL**

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The Effect of Weed Density, Root Senescence, and Egg Density on Western Corn Rootworm Larval Establishment, Survivorship, and Damage Potential

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ABSTRACT

Western corn rootworm, *Diabrotica virgifera virgifera* Leconte beetle emergence from and root damage to transgenic (Bt) maize (*Zea mays* L.) plants producing the Cry3Bb1 protein and isoline plants in the presence of varying *Setaria spp.* densities, egg densities, and *Setaria spp.* control times was evaluated in two separate field experiments. The nutritional value of senescing maize and *Setaria faberi* R.A.W. Herrm roots to neonate and second instar western corn rootworm larvae was evaluated in three separate greenhouse experiments. Greenhouse evaluations include glyphosate sprayed *S. faberi*, glyphosate sprayed maize, and maize severed below the growing point. Larval recovery, weight change, and adults emergence was monitored for each experiment. Results of the field experiment showed significantly greater root damage to the isoline hybrids than the transgenic hybrid. In one of sixteen comparisons with the transgenic hybrid, it produced significantly more beetles in the weedy plot than the weed-free plot. This is the first time this has been documented in a field situation. Results of the greenhouse experiments showed that once *S. faberi* was sprayed with glyphosate, it became nutritiously deficient to both neonate and second instar larvae within the first five days. Severed maize became nutritiously deficient between 5 and 10 d after the plant was killed. Glyphosate sprayed maize become nutritiously deficient between 10 and 15 d after the plants were sprayed. A minimal number of adults were recovered from any greenhouse treatment, except living maize or *S. faberi*.

CHAPTER 1

LITERATURE REVIEW

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, (Chrysomelidae, Coleoptera), is a pest in continuous maize (*Zea mays* L.) fields and in some first year maize fields. It is primarily a pest in its larval stages where it feeds almost exclusively on maize roots (Branson and Ortman 1970). Root damage from this pest can disrupt water relations (Riedell 1990), reduced nutrient uptake (Kahler et al. 1985), cause reductions in photosynthetic rate (Godfrey et al. 1993), and reduce plant stability (Spike and Tollefson 1989). In the adult stage, western corn rootworm beetles will feed on silks, maize pollen, and developing kernels on the ears (Gray and Tollefson 1988; Spike and Tollefson 1989). Unlike the larval stage, western corn rootworm beetles do not usually cause much damage or yield loss. Under extremely high populations, they can cause pollination problems.

LeConte (1868) first collected western corn rootworm in western Kansas in 1865. At this time, maize was not grown in the region according to a distribution map of maize in the 1860s (Weatherwax 1954). This suggests that maize was not the sole, original host of western corn rootworm although, it is by far the best host known to date (Branson and Ortman 1967 a, b, 1970, Clark and Hibbard 2004). Western corn rootworm was first recorded as a pest of maize in Colorado in 1909 (Gillette 1912). The first report of damage to maize roots was from northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, (Riley 1880).

Alternate Hosts

Branson and Ortman (1967b) showed for the first time that it is possible to rear western corn rootworm on non-maize plant species. In this experiment, they tested 22 grass species and six species produced adults: green foxtail (*Setaria viridis* L.), yellow foxtail [*Setaria lutescens* (Weigel)], Minter and Selkirk wheat (*Triticum aestivum* L.), Omugi barley (*Hordeum vulgare* L.) and Oahe intermediate wheatgrass [*Agropyron intermedium* (Host)]. Branson and Ortman (1967a) concluded that the fecundity of western corn rootworm beetles and the viability of their eggs do not appear to be reduced when they are reared as larvae on host other than maize. Branson and Ortman (1970) tested 44 grass species, 12 broadleaf weeds and 15 species of broadleaf crops. Of the 44 grass species tested, 18 supported western corn rootworm larvae for at least 10 days.

Clark and Hibbard (2004) tested 28 grassy species and 1 broadleaf weed. Of the 28 grass species, 13 were in common with those tested by the Branson and Ortman (1967b, 1970). This study had five larval sample dates (6, 10, 14, 20, 24 days after infestation) plus adult emergence. Of the 29 plant species evaluated, larvae survived at least 6 days on 27 of the plant species and 24 days on 23 of the plant species. There were 18 species that had larval development to second instars whereas larvae on 14 species developed to third instars. Six plant species that produced adults including maize, large crabgrass [*Digitaria sanguinalis* (L.)], barnyard grass [*Echinochloa crus-galli* (L.)], sand lovegrass [*Eragrostis trichodes* (Nutt.)], western wheatgrass [*Pascopyrum smithii* (Rydb.)] and Texas panicum [*Urochloa texana* (Buckley)]. Overall “maize had the highest larval recovery in all five samplings but 17 species also had larvae recovered at all samplings, two had recovery from four samplings, five from three samplings, one each for two sampling and one sampling(s) and three treatments had no larval recovery”

(Clark and Hibbard 2004). The results showed that the treatment by date significantly affected the head capsule width and mass accumulation because larvae grew at different rates for the species evaluated. In maize, most larvae had reached the third instar by the 14 d collection and the rest of the species did not reach this until 20 d or 24 d collection (Clark and Hibbard 2004). The maximum dry weight was reached on maize, reed canarygrass (*Phalaris arundinacea* L.), witchgrass (*Panicum capillare* L.), and wild proso millet (*Panicum miliaceum* L.) by day 20, while the remaining species did not reach maximum dry weight until collection day 24 (Clark and Hibbard 2004). This study contradicted Branson and Ortman studies (1967b, 1970) in a few areas. First of all, reed canarygrass and barnyard grass were not considered hosts by Branson and Ortman but were among the better host in Clark and Hibbard (2004). In addition, johnsongrass [*Sorghum halepense* (L.)], orchardgrass (*Dactylis glomerata* L.) and switchgrass (*Panicum virgatum* L.) were considered marginal host by Clark and Hibbard (2004) but were not considered host by Branson and Ortman (1967b, 1970).

Wilson and Hibbard (2004) broadened the plant species evaluated for western corn rootworm host suitability to include the remaining maize field grassy weeds not evaluated by Clark and Hibbard (2004) from a resistance management perspective. Of the 22 species evaluated, five were in common with those tested by Branson and Ortman (1967b, 1970) and four with Clark and Hibbard (2004). Seven days after infestation larvae were recovered from all the plant species and larvae were recovered on 18 of the 22 species on sample day 26. The plant species where the number of larvae recovered did not significantly differ from maize was fall panicum (*Panicum dichotomiflorum* Michx.), crabgrass, and western wheat grass (Wilson and Hibbard 2004). The head

capsule width (HCW) of larvae recovered from quackgrass (*Elytrigia repens* L.), Rhodes grass (*Chloris gayana* Kunth) and fall panicum these were not significantly different from larvae recovered from maize on all four-sample dates. Seven plant species had larval HCW not significantly different from maize (Wilson and Hibbard 2004). Third instars were recovered from 16 of 22 species evaluated. When looking at larval weight, larvae recovered from maize were found to be significantly heavier than larvae recovered from all other species on sample days 21 and 26 and when sample dates were combined. Adults were recovered on 10 of the 22 species.

Oyediran et al. (2004), evaluated 21 prairie grass species that were thought to be dominant species in the western Great Plains at the time that western corn rootworm beetle was first found. Fourteen of the 23 plant species (including maize and sorghum) produced adult rootworm beetles. There was no significant difference between the number of larvae recovered from maize, pubescent wheatgrass (*E. intermedia*), side-oats grama (*B. curtipendula*), slender wheatgrass [*Elymus trachycaulus* (Link.)], and western wheatgrass (Oyediran et al. 2004). The number of adults produced from pubescent wheat grass was not significantly different from the number produced from maize. The average dry weight of larvae varied significantly between sample dates but did not vary significantly between grass species (Oyediran et al. 2004). The HCW and average dry weight was not significantly different between maize, western wheatgrass, pubescent wheatgrass, slender wheatgrass, and galleta (*Pleuraphis jamesii* Torr.) (Oyediran et al. 2004).

Breitenbach et al. (2005) evaluated four weeds in Romania (*Setaria glauca*, *S. viridis*, *S. verticillata*, and *Sorghum halepense*), along with winter wheat (cultivar Josef)

and a maize control for survivability of western corn rootworm in a naturally infested field. The *Setaria* species all produced beetles, but emergence was delayed for about 14 days compared to maize (Breitenbach et al. 2005). Development for western corn rootworm larvae in the *Setaria* species was also reduced to about 15% compared to maize.

Alternate Hosts + Maize. Oyedrian et al. (2005) documented that more beetles were produced on MON863 + giant foxtail (*Setaria faberiana* R.A.W. Herrm) + large crabgrass than on MON863 alone or giant foxtail + large crabgrass alone in greenhouse trials. MON863 is a Bt event expressing the Cry3Bb1 insecticidal protein in the roots of selected transgenic maize produced by Monsanto Company. Beetle emergence was delayed in MON863 alone and MON863 + weeds but the later was delayed less in comparison to the isoline maize.

If similar data are documented in the field, this could lead to increased root damage on rootworm-resistant transgenic maize plants when weeds are killed. This could have a positive or negative impact on the development of resistance to transgenic maize in populations of the western corn rootworm. If the partial development on grassy weeds produces more susceptible adults in the transgenic maize field, that would delay the development of resistance. If partial development on transgenic maize selected for resistance, this could speed the development of resistance.

Johnson et. al. (1984) looked at impact of foxtail infestations on corn rootworm larvae on maize planted in early May and early June in 1981, 1982, and 1983. The main plot was planting date and the subplot was the presence or absence of a heavy foxtail infestation. Larval sampling and adult emergence were used to evaluate the effect of the

foxtail infestation on the western corn rootworm. Johnson et al. (1984) used a mixture of green foxtail and giant foxtail and did not use large crabgrass. The foxtail seed mixture was a 1:1 weight-to-weight mixture for green foxtail and giant foxtail and resulted in 1,400 giant and 3,000 green foxtail seeds per m² (Johnson et. al. 1984). The results showed a reduced larval population and reduced adult capture in the presence of the heavy foxtail infestation for all three years. The only contradiction was in the June 10 1983 planting in which the foxtail infested plot had 49% more western corn rootworm larvae per sample than the weed-free plots. Adult western corn rootworm emergence in the foxtail plots was delayed compared to the weed-free plots.

Ellsbury et al. (2005) studied the effects of yellow foxtail established in parallel rows to maize rows on western corn rootworm survival, root damage, lodging, biomass production and yield. This experiment took place in two different locations with two different soil types, which included Brandt silt clay loam and Vienna loam. In 1995 and 1996, results indicated a delay in time from infestation to 50% emergence of adults of 10 and 4 d respectively, in both soil types, in the presence of yellow foxtail. The root feeding damage was greatest in 1996 and root injury was greater when the foxtail band was positioned besides the maize row than when the foxtail band was positioned 10 cm from the maize row between the maize and egg infestation (Ellsbury et al. 2005). The HCW of beetles recovered above yellow foxtail bands were significantly smaller than the HCW of beetles recovered from maize. In 1995 the maize + yellow foxtail strips was lodged 65% less than from the maize only plots. In 1996 there was 50% fewer beetles recovered from the maize + yellow foxtail compared to plots where no yellow foxtail was planted. Stover biomass was greatest when yellow foxtail was not present than when it

was present. Yield was also significantly greater in the Vienna loam soil in 1995 when the foxtail was not present.

Alternate Hosts + Oviposition. In 1980 to 1983, Johnson and Turpin (1985) studied the effect of four different foxtail densities (400, 40, 4 and 0 seedlings per m of row) on the oviposition patterns of western corn rootworm and northern corn rootworm. It had been hypothesized by Kirk et al. (1968) that weedy areas in maize fields would probably receive more egg per unit area than clean areas. The results revealed no consistent relationship between foxtail densities and western corn rootworm and northern corn rootworm egg densities. It was also, noted, “root damage ratings from the years following oviposition were not significantly different among weed treatments in any tillage system in any year” (Johnson and Turpin 1985).

Alternate Host Phenology. Chege et al. (2005), looked at five different alternate hosts which included large crabgrass, giant foxtail, witchgrass, woolly cupgrass (*Eriochloa gracilis* Kunth), and green foxtail (maize was the control). In this experiment, each host plants were infested at eight different times from weeks four through eleven after planting using neonate western corn rootworm larvae. The number of larvae, larval weight, and HCW was recorded for the larvae recovered on three different recovery dates (7, 14 and 21 days) after infestation. The results showed that, in general, larvae gained more weight during early infestations than they did during later infestations (Chege et al. 2005). It was also noted that those infested after weeks 4-6 resulted in reduced larval weight gain and HCW increased more slowly. The hosts listed in the order of greatest weight gain to the least weight gain are maize, large crabgrass, giant foxtail, withchgrass, woolly crabgrass, and green foxtail. In other related field and greenhouse experiments,

western corn rootworm larvae could establish on late phenology maize and establish but adult emergence was greatly reduced (Bruce Hibbard, unpublished data).

Maize

Maize, as stated above, is the best-known host for western corn rootworm larval development. The developmental threshold of both maize and western corn rootworm are similar. Maize has a developmental threshold of 10°C and western corn rootworm has a developmental threshold of 11°C (Fisher et al. 1991). This synchronized development between the maize pest and the host plant may facilitate this relationship. Weeds in the genus *Setaria* on the other hand have a developmental threshold between 20 and 35°C (www.cdfa.ca.gov/phpps/ipc/weedinfo/setaria.htm).

In a normal year, maize root nodes 3 through 6 produce succulent new growth at the base of the maize plant during the time of western corn rootworm egg hatch and larval development (Riedell and Reese 1999). This leads to western corn rootworm damage on these specific nodes of the adventitious root system (Apple and Patel 1963; Riedel 1989). When root damage occurs, compensatory root growth (increased root volume, root length and root axes number) is common in the root node immediately above the root node damaged by western corn rootworm larvae (Riedell and Reese 1999). Riedell and Reese (1999) documented that severe larval rootworm damage (> 1 node of root axes destroyed) and lack of compensatory root growth causes reductions in shoot growth, leaf area and carbon dioxide assimilation. Maize plants with one node of roots damage, along with compensatory root growth, will grow and develop shoot organs in a manner very similar to undamaged plants. Plants with two nodes of roots damaged have

slower shoot growth and development than undamaged plants (Riedell and Reese, 1999). In plants severely damaged by the western corn rootworm, the shoot organ growth and development on the upper portion of the plant will be reduced (Riedell and Reese 1999; Riedell 1989; Gavloski et al. 1992).

Along with host phenology, the carbon/nitrogen plant ratio can affect the ability of western corn rootworm larvae to convert root biomass into insect biomass. The efficiency of western corn rootworm larvae in converting root biomass into insect biomass is positively correlated to the amount of nitrogen in the plant tissue (Mooser and Vidal 2004).

Maize Phenology. The time of egg hatch also affects the number of western corn rootworm larvae that survive past the first instar stage. Bergman and Turpin (1984) showed that early and late maize planting dates affect western corn rootworm numbers and growth stage. This field study took place near West Lafayette, Indiana in 1981 and 1982. In 1981, they had four planting dates: April 21, May 4, May 22, and June 4 and in 1982 there were three planting dates: April 26, May 10, and June 3. They found that planting date had no significant impact on the number of first instar western corn rootworm larvae collected in either year. However, fewer second and third instars, pupae and adults were collected in the later planting dates. Planting date also had a significant influence on the seasonal occurrence of second and third instars, pupae and adults in 1981 and only had a significant affect on the adults in 1982 (Bergman and Turpin 1984). The greatest effect was on adult emergence in both years, which showed a 13 to 14 day delay in emergence from the earliest to latest planting date. The data also suggested that the effects of delayed planting are manifested as the insects develop (Bergman and

Turpin 1984). Musick et al. (1980) also noted that late-planted maize had a reduced total emergence of western corn rootworm adults with peak emergence being delayed and drawn out over a longer period.

In a similar manuscript looking at the influence of planting date on western corn rootworm survival it was noted that the earliest planting dates yielded the most western corn rootworm adults and that the latest planting dates had the fewest (Fisher et al. 1991). There was no significant affect in adult fecundity between planting dates (Fisher et al. 1991). Significantly, fewer female western corn rootworm beetles were recorded at the later planting dates but the mean number of male per planting date remained the same (Fisher et al. 1991).

Stavisky and Davis (1997) studied the effect of maize hybrid maturity on western corn rootworm larval occurrence and adult emergence in a field study near Freeville, NY. The study took place in 1994 and 1995 and looked at the difference between three Pioneer hybrids 3979 (76 days to maturity), 3769 (97 days to maturity), and 3394 (110 days to maturity). In both years, 60% as many adults emerged from the early as from the late hybrid. Adult emergence was prolonged in the late hybrid. In 1994 and 1995, 90% emergence was approximately 10 and 6 days sooner in the early than in the late hybrid respectively. Larval sampling documented that late hatching larvae do poorly in the early maturing hybrid.

Insecticide

Hill et al. (1948) were the first to show control of western corn rootworm larvae by disking or plowing under a broadcast application of benzene hexachloride in early spring. Resistance to chlorinated hydrocarbons was first found in south central Nebraska

(Weekman 1961; Ball 1962). Weekman stated that 50% of the maize acres in this region that were treated with chlorinated hydrocarbons reported severe damage. It has also been shown that the western corn rootworm has developed resistance to organophosphate and carbamate insecticides in the same region when used for adult control (Meinke et al. 1998). Today there is a \$1 billion cost annually in control and yield loss from western corn rootworm. Up until 2003, organophosphate or pyrethroid soil insecticides were the only options to control western corn rootworm in continuous maize fields. In the past, few years transgenic Bt maize targeted toward corn rootworms have started to replace some soil insecticide acres (see section below).

Soil insecticides are used in continuous maize fields to reduce larval rootworm damage, prevent root lodging and yield loss but will not reduce survival of beetles to levels that prevent damaging infestations in the subsequent growing season (Sutter et al. 1991). The beetles that survive the soil insecticides have an equal or greater potential for egg production in the laboratory than those from fields without soil insecticides. In general, highly water-soluble soil insecticides are more effective in reducing corn rootworm populations, but these are at a greater risk of contaminating surface and ground water (Sutter et al. 1991).

Soil moisture has a dramatic effect on the protection provided by soil insecticides. In a five-year study (1980-1985), Sutter et al. (1989) found that the level of root protection was dependent on year (edaphic and environmental factors) and pest population density. Of the eight soil insecticides tested, terbufos (Counter 15g), ethoprop (MoCap 15g) and Chlorpyrifos (Lorsban 15g) had a residual near 100% mortality at day 94 after treatment in the years 1984 and 1985 (Sutter et al. 1989). Root ratings of these

three insecticides treatments were near the low end compared to the untreated. The other soil insecticides included in this study were carbofuran (Furadan 15ga), fonofos (Dyfonate 20g), phorate (Thimet 20g) and Isofenophos (Amaze 20g). In a similar study using the same soil insecticides, Sutter et al. (1990) look at the percentage of root lodging and yield protection of these insecticides with different western corn rootworm egg densities (0, 300, 600, 1200, and 2400 eggs per 30.5cm of row). They found that at the 300 and 600 egg densities, there was not a significant difference in the yield of the insecticide treated maize verses the controls. At the 1200 and 2400 eggs densities, the yields in the plots where insecticides were used were significantly higher compared to the control plots. They also did not find any significant correlation between root damage ratings and yield of plants treated with any of the soil insecticides. With these results, the use of root damage ratings by themselves to evaluate soil insecticides in comparison with yield may be questionable. It has also noted that under good growing conditions a root rating of one, on the current Iowa State University (ISU) scale, must be reached before yield losses would be minimized by insecticides (Gray and Steffey 1998; Sutter 1990).

Wright et al. (2000) looked at six soil insecticides and their root protection against a western corn rootworm population resistant to methyl parathion and carbaryl and a susceptible population. The resistant population was selected on adults through beetle spray programs (Meinke et al. 1998). When testing the resistant population verses the susceptible population in a laboratory study, they found that 3rd instars of the resistant population were 16.4 times as resistant to methyl parathion (O-methyl-substituted organophosphate insecticides) using topical applications. However, they found that O-ethyl-substituted insecticides (terbufos and chlorypyrifos) were not as highly tolerated in

the resistant population. In field trials, the following insecticides: terbufos (Counter 20CR, Counter 15G, American Cyanamid, Parsippany, NJ); chlorpyrifos (Lorsban 15 G, Dow Agrosience, Indianapolis, IN); tebufos + cyfluthrin (Aztec 2.1 G, Bayer, Kansas City, MO); fonofos (Dyfonate 15 G, Zeneca Ag Products, Wilmington, DE); chlorethoxyphos (Fortress 2.5 G, Fortress 5 G, DuPont, Wilmington, DE) were used to test root ratings. They found that Counter 15 G, Counter 20CR and Lorsban 15 G resulted in the lowest root ratings. Force 3 G resulted in significantly higher root injury but significantly better than the control. Aztec 2.1 G, Fortress 5G and Dyfonate 15G were significantly better than the control but significantly less than Counter 15 G, Counter 20CR and Lorsban 15 G. Results showed that organophosphate (terbufos and chlorpyrifos) soil insecticides are still highly effective when used at planting time against methyl parathion resistant larval corn rootworm (Wright et al. 2000)

Transgenic Maize. On 25 February 2003, MON863 (Monsanto Company, St. Louis MO), which expresses the Cry3Bb1 endotoxin, was registered for commercial use. This was the first registered Bt (*Bacillus thuringiensis* Berliner) product used in transgenic maize for the control of western corn rootworm larvae and is referred to as YieldGard Rootworm. Bt is a common soil microorganism that naturally produces Bt proteins, which in this case is toxic to western corn rootworm. Different strains produce several different classes of insecticidal proteins with more than 100 different insecticidal genes. “The largest class consists of the delta-endotoxins, which often are produced as a 125-140 kDa protein precursor that is solubilized and proteolytically processed in the insect gut to yield a 55-75 kDa biologically active core protein” (Moellenbeck et al.

2001). Besides maize, Bt insecticidal proteins have been transformed into cotton, potatoes, rice and other crops.

Bt organisms also produce vegetative expressed insecticidal proteins, which include binary toxins Vip 1 and Vip 2 with coleopteran specificity and Vip 3 with lepidopteran specificity (Moellenbeck et al. 2001).

Dow AgroScience LLC and Pioneer have transformed maize to express the Cry34Ab1 and Cry35Ab1 proteins from the Bt strain 149B1 in transgenic maize plants (Storer et al., 2006). Storer et al. (2006) stated, “These two proteins act together as a binary insecticidal protein that is effective against corn rootworm species.” This Bt strain is comprised of two proteins with a molecular mass of approximately 14 kDa and 44kDa. “Phosphinothricin acetyltransferase was used as the selectable marker conferring resistance to the herbicides glufosinate (organophosphorus herbicides) and bialaphos” (Moellenbeck et al. 2001). Because the PS149B1 strain has oral toxicity to the rootworm larvae, some feeding must occur first for the larvae to be affected. The midgut epithelium of corn rootworm larvae is the site that is affected by the PS149B1 proteins, as is the case with Cry-1 and Vip3-type insecticidal proteins (Moellenbeck et al. 2001). This product was approved in 2005 by the environmental protection agency (EPA) and is referred to under the trade name of Herculex RW. In a field experiment by Storer et al. (2006) it was noted that this Cry34/35Ab1 expressing event 59122 caused 99.1 to 99.98 mortality of western corn rootworm when the density dependent mortality of the adults emergence numbers were taken into effect. With this transgenic maize, it was found that there was a short delay in time to 50% adult emergence but emergence was complete at about the same time as the control.

In 2006, Syngenta gained approval for their first transgenic maize resistant to rootworm larvae. This MIR604 maize event contains the modified full-length Cry3Aa gene from Bt. This transgenic maize is being made available to producers in 2007.

With the advent of transgenic maize that is resistant to western corn rootworm larval feeding, a study by Hibbard et al. (2005) was conducted to describe the effect of Cry3Bb1 expressing transgenic maize on plant-to-plant movement. This was a two-year study conducted in 2001 and 2002. In 2002, they found that significantly more neonates were recovered on nontransgenic maize, which was adjacent to infested transgenic maize on their second larval recovery sample date. This data implied a preference for nontransgenic maize by western corn rootworm larvae when given a choice. It was noted that there was little movement from non-transgenic maize to transgenic maize (Hibbard et al. 2005).

Seed Treatments. Along with the addition of transgenic maize against rootworm larvae, there has also been a new class of insecticides used as seed treatments to protect against western corn rootworm feeding. This class of insecticides is the neonicotinoids and seed treatments have been manufactured from Gufafson (Bayer CropScience) and Syngenta company. The active ingredient in Gufafson's seed treatment is clothianidin and the rootworm labeled products trade name is Poncho[®] 1250. Syngenta's product is Cruiser 5FS[®] and the active ingredient is thiamethoxam.

Biology/Adult

Adult western corn rootworm when emerging from the pupae stage undergoes a phenomenon known as protandry. This is when males emerge before the females. This is common in insects that have discrete generations with females that mate once soon

after emergence and males that mate repeatedly (Wilklund & Fagerstrom 1977; Bulmer 1983) all of which western corn rootworm fit (Branson et al., 1977; Guss, 1976). This has been shown to be an advantage in male competition for mates, which by emerging earlier increases the number of receptive females a male western corn rootworm beetle, encounters (Wilklund & Fagerstrom 1977). This phenomenon occurs in western corn rootworm because of a later egg hatch and longer post-hatch development for female western corn rootworm beetles. This same trend was also found to be the case in field situations in which male western corn rootworm beetles predominate for 40% of the emergence period (Fisher 1984).

Quiring and Timmins (1990) described the adult life stages for both male and female western corn rootworm beetles. The male western corn rootworm adult stage can be split into two stages: teneral and non-teneral. The teneral stage is the stage that follows eclosion from the pupal cell and can be described as a recently molted condition, indicated by a soft, pale-colored exoskeleton. Quiring and Timmins (1990) determined that depending on the size of the male western corn rootworm they are from 2 to 3.5 d old at the time of first mating. The mating stage lasts around 42 days (Quiring and Timmins 1990). Most females mate within the first 24 hours after emergence. Adult female western corn rootworm developmental period can be split into five stages: 1) teneral adult period (1d), 2) preoviposition (13d which includes teneral period), 3) first oviposition period (60d), 4) second nonoviposition period (which continues until the second mating) and 5) second oviposition period (which starts after the second mating) (Branson and Johnson 1972; Hill 1975; Fisher et al. 1991). Most females do not live long enough for second mating and it is not known the extent, if any, that this occurs in the

field. Onsted et. al (2001) description of the 13 d preoviposition period was similar to Branson and Johnson (1972) 14.3d preoviposition period but was shorter than the 23.3 to 29.0d period described by Kuhlman (1970) and 20-23d period described by Short and Hill 1972. Peak oviposition is about one to two weeks after the onset of oviposition and then declines after that (Kuhlman et al. 1970, Branson and Johnson 1972, and Cates 1969). Branson and Johnson (1972) manuscript is in agreement with Onsted et al. (2001) that female western corn rootworm beetles will oviposition for 60 d and only need one mating per female per season.

The sexual activity of western corn rootworm adult varies between males and female beetles with the female beetles being mainly monogamous and the male beetles mating with multiple females. Hammock (1995) stated that females can release pheromones and call for males for at least 3 day and within a 24 hour period after insemination will quit calling. Insemination causes females to become either unattractive or unreceptive to males (Hill 1975 and Branson et al. 1977). Male western corn rootworm beetles have been described to mate up to 8 to 11 times during their lifetime (Quiring and Timmins 1990; Branson et al. 1977). If female western corn rootworm beetles mate a second time it will have to be with a different, younger male because male western corn rootworm beetles life span is approximately 44d.

The number of western corn rootworm eggs that hatch and make it to adulthood can depend on many factors, one of which is population density. In artificially infested field trials, as the egg density increases from a low to a very high infestation the percentage of larvae that survive to adults is significantly reduced and there is an increase in time required to reach maturation (Branson and Sutter 1985; Weiss et al. 1985, Onstad

et al. 2006). This is believed to have resulted because of reduced feeding sites and inadequate food supply (Branson and Sutter 1985). Elliott et al. (1989) showed that at 300 and 600 eggs/0.3m of row that 4.8% of eggs survived to adults and at 2400 eggs/0.3m of row 1.5% of the eggs survived to adulthood. Branson and Sutter (1985) documented that high densities of eggs resulted in greater damage but a lower percentage of adults being produced because of density-dependent mortality.

Adult Emergence. In western corn rootworm adult emergence studies that take place in the field, it has been a common practice to sever maize plants close to the ground to simplify trap design for adult beetle recovery. Fisher (1984) showed that the number of adults recovered from severed and intact maize was not significantly affected in both artificial and natural infestation when the timing of severing was delayed until pupation. In the severed plots emergence was accelerated.

Fecundity. Ball (1957) in a study showed the lifespan of female western corn rootworm at 56.6 days and male at 44.7 days. Female beetles oviposit on average from 372 to 418 eggs. Branson and Johnson (1972) found the average number of eggs oviposited per female to be around 1023 eggs/female. The number of eggs oviposited per female can be highly variable depending on how the adults were reared.

Population Estimates. Scouting for adults in maize fields is a common practice used by researchers, consultants, and a few producers to predict the next year's western corn rootworm pressure when in a continuous maize production system. Park and Tollefson (2005) noted that the best prediction of adult emergence the following year in a continuous maize field is adult counts in the ear zone at peak population densities during the present year. Peak populations are three weeks after the first beetle was found and

ear zone refers to “the area from and including the upper surface of the leaf below the ear through the lower surface of the leaf above the ear” (Parks and Tollefson 2005). The economic injury level (EIL) used for this study was 0.35 adults per ear zone when the counts were average over a 10 plant counts. Scouting the fields using this method will let producers know where there high and low populations of western corn rootworm adults are along with next years highest rootworm pressure. This will allow producers to place their refuge acres in the lowest rootworm pressure area and then plant their Bt maize in the higher beetle count area to maximize their returns for buying and planting the resistant maize.

Biology/Larvae

Western corn rootworm larvae use carbon dioxide given off from the growing roots of maize plants to locate their host (Strnad et al. 1986; Hibbard and Bjostad 1988; Bernklau and Bjostad 1998 a, b). In a behavior bioassay, it has been shown that elevated levels of carbon dioxide can prevent western corn rootworm larvae from locating maize roots (Bernklau et al. 2004) and carbon dioxide generating materials (Bernklau and Bjostad 1998a). Carbon dioxide, which is normally an attractant to western corn rootworm larvae, is not involved in host recognition. Neonate larvae that contacted a host root (maize or wheat) and then removed had a localized search behavior (Strnad and Dunn 1990). If exposed and removed from nonhosts or a control, the neonate larvae had a ranging search behavior. Ranging is a relatively straight form of movement at a relatively faster pace and localized search is characterized by increased turning rate and decreased locomotory rate (Bell 1985). Although giant foxtail has been shown to be a marginal host, it did not stimulate localized search in neonate western corn rootworm

(Strnad and Dunn 1990). Other plant species tested that did not stimulate localized search include oats and soybeans (Strnad and Dunn 1990).

Developmental Time. Insects and plants are similar in the fact that they are dependant on the temperature of their surroundings. Since western corn rootworm larvae spend all of their developmental stages in the soil, it is possible to calculate their developmental stage just by knowing the growing degree-days (GDD). This is not an exact science unless you know the exact depth of the eggs or larvae in question. GDD are calculated using the equation $GDD = (High\ temperature - low\ temperature) / 2 - baseline\ temperature$. For western corn rootworm, the baseline temperature is 11°C and any lower temperature recorded for the day is set to 11°C for the low temperature. The optimum temperature for maximum rate of western corn rootworm development is 30°C so the maximum temperature is capped at 30°C (Fisher 1986; Jackson and Elliott 1988). Fisher (1987) developed a linear equation that would predict the 50% larval occurrence of each stage of western corn rootworm larvae. He predicted first instars to take 74.0 GDD, 2nd instars: 150.5 GDD and 3rd instars: 247.2 GDD (Fisher 1987). This experiment took place in a controlled greenhouse environment with 26±5°C pot soil temperature, with the temperature being recorded at 8cm depth, 45-65% relative humidity and a 14:10 [L:D] photoperiod. Pupae developmental threshold were found to be around 15°C (Fisher 1986).

Branson (1987) tested the different developmental times between male and female western corn rootworm by hatching 426 western corn rootworm eggs at 24-25°C under a 14:10 L:D photoperiod. Male's egg hatch dominated the first two days, there was a mixed hatch for third and fourth day and females dominated the fifth to tenth day of the

hatching period (Branson 1987). With the longer post hatch development of female western corn rootworm larvae males dominated the first five days of beetle emergence and females dominated the last nine (Branson 1987).

Larval development time between sexes is also apparent and males will develop 0.9 to 3.1 days faster when reared at constant temperatures between 15 and 31.5°C (Jackson and Elliot 1988, Kuhlman et al. 1970). Temperatures between 21 and 30°C yielded the most adults, widest HCW and the lowest proportion of deformed wings (Jackson and Elliot 1988). Average HCW declined for western corn rootworm larvae reared below 21°C. As temperature increased, the amount of time in the third instar stage increased and decreased in the first instar stage relative to the total time from hatch to adulthood (Jackson & Elliot 1988). Time spent in percentage wise in each stage averaged over the whole temperature range (15 to 31.5°C) was 1st = 18%, 2nd = 16%, 3rd = 36% and 30% for the pupal stage (Jackson and Elliot 1988).

Instar Determination. The HCW of western corn rootworm larvae is the only indicator of larval instar stages. Western corn rootworm larval HCW vary some with first instars ranging from 0.2 to 0.26 mm, second instars range from 0.3-0.4 mm and third instars range from 0.44-0.56 mm (Hammack et al. 2003). Hammack et al. (2003) also stated that maize root systems by themselves produce an unbiased estimate of western corn rootworm larval development stages compared with soil samples and root system as long as most larvae are in early larval stages. Head capsule width of western corn rootworm larvae will vary some though as stated above especially in cases of high rootworm densities. Branson and Sutter (1985) found in an experiment in which maize

plants were infested with 0, 300, 600, 1200, and 2400 eggs per 30.5 cm row that HCW significantly decreased as egg density increased up to 1200 egg rate.

Root Damage. Oleson et al. (2005) came up with an improved node-injury scale used by researchers to rate damage caused to the maize root systems by western corn rootworm. The range of this scale is 0 to 3 and has a linear relationship between numerical scale and the amount of root injured. A root is considered pruned if it is eaten to 3.8 cm of the stalk and or if the brace root starts above ground and the pruned root is pruned 3.8 cm from the soil surface (Oleson et al. 2005). To calculate the root rating score count the number of pruned roots in a node and divide it by the number of total roots in that node and add to the repeated process with the other three nodes (Oleson et al. 2005). For clarification, one node pruned would equal a one on the scale and three nodes pruned would equal a three. When sampling a naturally infested field using this scale it is recommended to skip four to five plants in a row to avoid sampling redundancy.

Damage patterns in naturally infested field are not uniform. Park and Tollefson (2005) stated that dispersion patterns of root injury by corn rootworm were random at large scale fields and aggregated in the moderate and small scale fields. The size of the fields included: 8 hectares for the large scale, 2000 to 2500 m² for the moderate scale and 25m² for the small scale. They stated that the minimum distance to obtain independent samples in common insecticidal trials is one meter apart within a row.

The amount of damage caused by western corn rootworm larvae can vary depending on the source of the eggs used (in artificial infestations) and the food the adults were reared on. Fisher et al. (1986) showed that damage and yield loss caused by

larvae from adults that were feed artificial diet were significantly greater than the larvae from adults that were feed natural maize plants at least at the 300 and 600 eggs per 30.5cm row. The damaged caused by western corn rootworm diapausing and non-diapausing strains has also been shown to have varying results (Hibbard et al. 1999, Branson et al. 1981). It is believed that soil temperature is the main factor and nondiapausing eggs will work well in situations when the soil temperature is high enough to enable the eggs to develop and hatch more quickly (Hibbard et al. 1999).

Larval Sampling. Larval recovery of western corn rootworm larvae is primary done using three methods, which include visual searching, washing-sieving-flotation and Tullgren funnel (Bergman et al. 1981). Bergman et al. (1981) tested these methods using fresh, cooler-stored or frozen samples (visual and washing-sieving-flotation only) at two different soil sample sizes. They found that frozen samples that were processed using the washing-sieving-flotation technique had the highest number of larvae recovered. They also discovered that using a soil sampling unit, which is 18 cm² by 10cm deep, was more efficient than a core, which was 10 cm in diameter by 10 cm deep. The processing time of the 18 cm square is over twice as long compared to the core (19 min vs. 8 min) but the accuracy of the 18 cm square samples is much greater and thus fewer samples need to be taken. Another larval recovery process was described in Fisher (1981) in which he developed a soil washing apparatus used to extract 2nd and 3rd western corn rootworm instars from soil samples. This apparatus had 93.4% accuracy in recovering larvae and two people could process 100, 1.4-liter soil samples per day. Hibbard et al. (2004) described a technique for recovering larvae from whole root balls by hanging them in a

greenhouse over water pans. This technique is a relatively efficient method for recovery larvae from the field.

Host-to-Host Movement. Larval movement, once they have established on a host plant, has been documented and adds to the western corn rootworm insect resistance management in relation to the recently released transgenic maize plants against western corn rootworm larvae. Hibbard et al. (2003) documented the movement and root damage of western corn rootworm larvae in the field from single isoline maize plants that were infested with 2000 eggs/plant (1500 viable eggs). Eleven plants including the infested plant were sampled which included the three closest plants (P1, P2 and P3) on each side of the infested plant within the same row and two plants from each adjacent row directly across from the infested plant. The results from this experiment showed that “damage levels were the highest on the infested plant and significantly decreased on more distal plants” (Hibbard et al. 2003). In three of the four experiments, larvae recovered from P3 had a significantly greater average larval weight than those recovered from the infested plant on the 3rd sampling date (5 total sample dates). Although larvae were found to move up to three plants down the row, larval movement after establishment was clearly documented in only two of eight experiments in two years. In a similar experiment looking at how egg density had an impact on larval movement after establishment, it was found that low to moderate infestations had little to no movement from the infested plant and that high infestations had significant movement from the damaged to undamaged plants down the row (Hibbard et al. 2004). The egg densities used in this experiment were 100, 200, 400, 800, 1600, and 3200 eggs/plant. These two experiments documented

post-establishment larval movement as far as 61 cm, but statistically significant larval movement was documented across a 76 cm row in the 2004 manuscript under very high infestation rates (Hibbard et al. 2003; Hibbard et al. 2004).

Soil bulk density can also affect western corn rootworm larval movement along with different soil types. Strnad and Bergman (1987) looked at this in three different soil types under different soil bulk densities varying from 1.1 to 1.5 g/cm³. In sandy loam soil they found that western corn rootworm larvae could move < 5 cm in a 1.1 g/cm³ soil bulk density. In the silt loam soil they found larval movement to be from 6 to 15cm in 1.1 g/cm³ soil bulk density and < 5cm in the 1.3g/cm³ soil bulk density. In sand larval movement was not restricted in the 1.1 g/cm³ soil bulk density but was restricted to < 20cm in the 1.3 g/cm³ and < 5cm in the 1.5 g/cm³ soil bulk densities. Maize root growth at 1.4 g/cm³ soil bulk density continues satisfactory (Waldren 1983). Therefore, some western corn rootworm larval survival/establishment may depend on root growth rather than larval movement at different soil bulk densities.

Western corn rootworm larvae survivability decreases rapidly with the increasing amount of time it takes the larvae to find its host. Branson (1989) found that after one day of starvation larvae had a 45% reduction in adult emergence; at two days of starvation there was a 64% reduction in adult emergence; and a 91% reduction in adult emergence at 3 days.

Diabrotica Identification. Larval identification of corn rootworm down to species in Missouri can be a challenging task. First of all the larvae need to be fairly mature (2nd to 3rd instars) mostly for the ease of handling. The three species that we have in Missouri that attack maize roots are western corn rootworm, northern corn rootworm

and southern corn rootworm (SCR) (*Diabrotica undecimpunctata howardi* Barber). SCR differs morphologically from western corn rootworm and northern corn rootworm by the presence of urogomphi, which are small protrusions on the posterior margin of the anal plate (Mendoza and Peters 1964). Western corn rootworm differ from SCR by “a definite notch in the darkened area of the anterior margin of the anal plate and a sclerotized band underneath the posterior edge of the anal plate (Mendoza and Peters 1964). If the corn rootworm larvae have neither of these markings then they are northern corn rootworm. However, Krysan et al. (1986) noted that because of some variability between distinguishing features of the larvae of *D. v. virgifera* and *D. barberi* proper identification is not possible. Clark et al. (2001) found that it is possible to distinguish between several *Diabrotica* species using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) of the COI gene. This key is to be used as a supplement to current morphological keys to distinguish between morphologically similar species.

Biology/Egg

Western corn rootworm eggs used in rootworm research come from two different types of western corn rootworm, which include diapausing and non-diapausing populations. Diapausing strains are the natural/wild strains found in maize fields throughout the Midwest states. The non-diapausing strains were bred for in laboratory. Diapause as defined by Beck (1968) “is a genetically determined state of suppressed development... which most typically begins long before the onset of unfavorable physical conditions....” In a manuscript on the thermal requirement and hatching patterns of western corn rootworm eggs Levine et al. (1992) noted that for western corn

rootworm populations in the 40°N latitude that the diapause requirement for eggs is about 130-142 days. Levine et al. (1992) also documented the first prolonged diapause in western corn rootworm wild populations that required two winters to hatch.

Western corn rootworm populations from different latitudes have been shown to have differences in the time required for eggs to hatch from these populations. Wilde et al. (1972) suggested that eggs from northern latitudes (South Dakota and Minnesota) tend to hatch earlier than eggs from southern latitudes (Iowa, Nebraska, Missouri and Kansas) at controlled temperatures and that physiological differences probably exist. Differences in hatching times in Branson (1987) and Levine et al. (1992) from South Dakota and Illinois populations respectively adds support to this hypothesis.

Quiescence is different than diapause in it is a direct response to unfavorable environmental forces resulting in a retardation or cessation of development that is immediately reversible upon the return of adequate conditions. Krysan (1978) found that post diapausing eggs held at 25°C and 96%RH (relative humidity) underwent some development then became quiescence until these eggs came in contact with moisture. Once this happened they hatched within 13 days. He also noted that eggs in this condition took longer to hatch than eggs held at 25°C and moisture, which was 8 days compared to 13 days. Once western corn rootworm eggs have undergone diapause they become turgid which is the result of water uptake (Krysan 1978). Eggs which became turgid within the first few days after being removed from chilled situations (laboratory) were abnormal or dead eggs because water uptake is not normal during diapause (Krysan 1978).

Artificial Infestation. Natural infestations of rootworm eggs lack uniformity and density estimates of the number of rootworm eggs are difficult to obtain and these reasons add to the need for artificial infestation in research situations. Sutter and Branson (1986) described artificial infestation of western corn rootworm eggs as early as 1964, when F. F. Dicke (USDA-ARS) mixed fine, dry soil with eggs and applied the mixture in the field. Chiang et al. (1975), described this technique in conjunction with their enhancement of a mechanical device to apply the soil-egg mixture. Palmer et al. (1977) improved this technique by using a dilute solution of agar and water to suspend the western corn rootworm eggs. Sutter and Branson (1980) developed a tractor-mounted infestation device for infesting the eggs and Moellenbeck et al. (1994) modified this device for further improvements.

Wilson et al. (2006) infested two egg densities (400 and 800 eggs/plant) from 1, 2, 4, 8 or 16 infestation points that were 12.7 cm from the plant. They showed that root ratings increased from 0.12 ± 0.03 to 0.74 ± 0.3 and 0.18 ± 0.11 to 0.56 ± 0.15 on the 0 to 3 scale when the number of infestation points increased from 1 to 16 for the 400 and 800 egg densities respectively. Adult emergence was also monitored for this experiment but was not significantly affected by the different number of point source infestations.

Consistent differences between larval establishment in greenhouse and field experiments have been documented. Two to seven percent of viable western corn rootworm eggs will establish on maize plants in field situations (Branson et al. 1980; Branson and Sutter 1985; Fisher 1985; Elliot et al. 1989; Sutter et al. 1991; Hibbard et al. 2004). In greenhouse situations, there is a 5 to 10 fold increase in larval establishment

and a 30 to 50% increase in larval establishment and recovery (Weiss et al. 1985; Clark and Hibbard 2004; Wilson and Hibbard 2004; Oyediran et al. 2004).

The timing of when to infest field trials, to maximize the damage, with western corn rootworm egg has had some differing results in different environments. Branson and Sutter (1986) found that western corn rootworm root damage to maize roots was significantly greater when eggs were infested after plants emerged compared to infesting at planting time, or when multiple infestations are used (half at planting and half shortly after the plants have emerged). Hibbard et al. (1999) found that planting time infestation had significantly more damage than 2-leaf maize, and the 2-leaf infested maize had significantly more damage than the 5-leaf maize.

Egg Survivability. Tillage practices in continuous maize field have also been shown to have some influence on the survival and damage effects of western corn rootworm eggs. In an article that looked at the differences between no-till, chisel plow and moldboard-plow tillage practices, Johnson and Turpin (1985) found that in two of the three years (1981 and 1983), no-till root ratings were significantly less than chisel plow plots. In 1982, moldboard-plow plots had significantly less damage than both chisel-plow and no-till plots. In all three years though, chisel plow had the highest western corn rootworm root damage.

Temperatures during the diapausing state can have an affect on the survivability of the eggs. Ellsbury and Lee (2004) described the super-cooling capacity of western corn rootworm eggs and found that minimum temperatures below -10°C and approaching -13°C during a five week period in December to January caused a significant mortality of western corn rootworm eggs which was consistent with Gustin (1981). In laboratory

conditions Ellsbury and Lee (2004) found the mean supercooling point for western corn rootworm eggs in wet, moist and dry soils to be $-21.8 \pm 1.1^{\circ}\text{C}$, $-21.7 \pm 1.1^{\circ}\text{C}$ and $-27.8 \pm 0.3^{\circ}\text{C}$ respectively. They also found that northern corn rootworm can withstand lower temperatures but are more vulnerable to moisture when it comes to egg hatching.

CHAPTER 2

Introduction

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a major pest of continuous maize, *Zea mays* L., in Missouri and throughout the Midwest Corn Belt in the United States. It has been estimated the cost of control and yield loss from this pest has cost producers and estimated 1 billion dollars annually (Metcalf 1986). In central Missouri, the pest is controlled by three primary methods that include crop rotation, soil insecticides, and in the past few years, the addition of transgenic hybrids that produce insecticidal proteins derived from *Bacillus thuringiensis* (Bt) Berliner in the roots (Moellenbeck et al 2001, Vaughn et al 2005). The addition of these transgenic hybrids has increased research interest in western corn rootworm especially in insect resistant management (IRM).

In 2003, the first commercially available transgenic trait expressing Cry3Bb1 protein (YieldGard Rootworm, Monsanto Company, St. Louis MO) targeting pest *Diabrotica* was made available in selected corn hybrids also known as MON863. The YieldGard rootworm trait provides an effective dose against first instar larvae but it is less efficacious against larvae that have developed past the instar stage (EPA SAP 2002). This information has led to some recent research on the ability of western corn rootworm larvae to utilize hosts beyond maize (Clark and Hibbard 2004, Oyediran et al. 2004, Wilson and Hibbard 2004). Their results indicated that 57 of the 60 grass species evaluated could support western corn rootworm development to at least the second instar stage. The ability of the western corn rootworm larvae to utilize grassy weeds as a way to increase survival in the presence of the transgenic hybrid (MON863) expressing the

Cry3Bb1 protein was tested by Oyediran et al. (2005, 2007). Oyediran et al. (2005) demonstrated that the availability of grassy weeds when planted in close proximity to a MON863 hybrid can enhance western corn rootworm beetle emergence in greenhouse experiments. This suggests that some individual larvae gained enough mass on the available grassy weeds to overcome the dose being expressed in the MON863 hybrid being examined. Oyediran et al. (2007) evaluated similar combinations in a field setting, but the number of adults produced from MON863 was not significantly greater when weeds were present than when they were not.

Since the results of interactions studies involving MON863 and alternate hosts were different between the greenhouse and the field (Oyediran et al. 2005, 2007), the objective of the current experiments were to evaluate different weed densities and different egg densities in the field to determine if these factors have an impact on adult emergence from and damage to MON863 in the field.

2.1 Material and Methods

Experimental Site and Design. *Experiment 1.* This study was conducted in 2005 and 2006 at the University of Missouri, Bradford Research and Extension Center, located 9.6 km east of Columbia, MO. The soil type at this location is a Mexico silt loam comprised of 12.5% sand, 65% silt, and 22.5% clay as determined by the University of Missouri Soil Testing Facility, Columbia, MO. Each site had been planted to soybeans, *Glycine max* L., in the year prior to the initiation of each study so an absence of feral western corn rootworm eggs at each site was assumed.

In 2005 and 2006, the study was set up as a two × two × three (green foxtail (*Setaria viridis* (L.)) density × egg density × insect management tactic) factorial arrangement in a randomized complete block split-split plot design. The main plot was the presence or absence of green foxtail. The subplot was western corn rootworm egg infestation levels (2000 and 7025 viable eggs/meter). The sub sub-plot was the use of three insect management tactics; a transgenic (Bt) hybrid, an isoline hybrid (same base genetics) plus tefluthrin in a T-band at a linear rate of 0.014 g (AI) / m (Force 3G, Syngenta Corporation), and the isoline hybrid without an insecticide. The study had twelve treatments and four replications. Each main plot was 3.05 m long × 28.19 m wide with a 1.52 m alley between replications. Each replication was randomly split, with one half having green foxtail and the other side kept weed free. Within each of the weedy and weed-free portions, there were six treatments consisting of two maize rows and one border row between each treatment. So, each insect management tactic by absence/presence of green foxtail was challenged by two western corn rootworm egg infestation rates.

Experiment 2. This study was located approximately 16 km northwest of Higginsville, in Lafayette County, Missouri in 2004 and 0.8 km North-West of the Saline County fairgrounds near Marshall, MO in 2006. The 2004 location soil type was Marshall silt-loam with a composition of 15% sand, 67.5% silt, and 17.5% clay as determined by the University of Missouri Soil Testing Facility. The 2004 and 2006 locations had been continuously planted to maize for 6 and more than 10 years respectively with a background population of western corn rootworm that caused economic damage in the year prior to conducting each study.

This study was set up as a $2 \times 3 \times 3$ (insect management tactic \times giant foxtail density (*Setaria faberi* R.A.W. Herrm.) \times giant foxtail control times) factorial arrangement of a randomized complete block design. The insect management factors were MON863 and isoline maize hybrids, the giant foxtail, *Setaria faberi* Herrm, densities (0, 9.1, and 23.7 plants / m²), and three giant foxtail control times (3, 7, and 14 d) after initial detection of egg hatch. The study was replicated five times with eighteen factorial combinations per replication. Each factorial arrangement consisted of two maize rows, 3.05 m in length with 76 cm row spacing, and a 1.52 m alley free of vegetation between each treatment. Two border rows were planted between each replication.

Maize and Foxtail Planting. In both experiments, the field sites were tilled shortly before the fields were planted. Maize was planted using a 2-row planter (John Deere MaxEmergence, Deere and Company, Moline IL) at a rate of 67,500 seeds per ha. Maize was planted at a seed depth of 4.5 cm, a row spacing of 76 cm and two rows per treatment.

Experiment 1. One and a half liters of green foxtail seed (Azlin Seed Co., Leland, MS) was sown per replication on 20 April 2005 and 17 April 2006 using a hand-held broadcast spreader (model 126, Spyker Spreaders, LLC. Urbana, IN) and incorporated lightly afterwards with a harrow pulled by a four wheel all terrain vehicle (Table 1). Maize was planted on 3 May 2005 and 25 April 2006 (Table 1).

In 2005, a very heavy green foxtail stand (approximately 1017 plants / m²) was achieved and because of the even distribution of plants throughout the four reps, the stand

was left untouched. In 2006, a similar initial green foxtail stand was achieved but was thinned to a more moderate density of 9.1 plants / m² on 5 June 2006, which, was achieved through hand weeding. In 2006, each foxtail treatment had 42 foxtail plants, with 11 strategically spaced within 38 cm of each maize row, and 20 spaced-out between the two maize rows.

The transgenic hybrid expressing the Cry3Bb1 protein in 2005 was DKC 60-12 and its near-isoline was DKC 60-15 (Monsanto Company). In 2006 the transgenic hybrid expressing the Cry3Bb1 protein was DKC 60-18 and its near-isoline was DKC 60-19 (Monsanto Company). All hybrids in 2006 were glyphosate resistant and these hybrids had the same base genetics as the 2005 hybrids.

Experiment 2. Maize was planted on 7 May 2004 and 21 April 2006 (Table 1). In 2004, the giant foxtail was a natural infestation. In 2006, the giant foxtail seed (Azlin Seed Co., Leland, MS) was planted the same day as the maize (Table 1). The foxtail was hand sowed and lightly raked in, to ensure proper seed-to-soil contact. The amount of seed used for the low density was 85 ml of seed per plot and the high density was 170 ml of seed per plot. The dimensions of each plot seeded were 1.52 m (width) × 3.05 m (length).

In 2004, the high (23.7 plants / m²) giant foxtail density was allowed to stand as they were because they were right near the high-density limit. The moderate (9.1 plants / m²) giant foxtail density was thinned down by hand on 20 May 2004. In 2006, the high and moderate giant foxtail densities were thinned down by hand to desired densities from May 30 to June 2.

In 2004, the transgenic hybrid expressing the Cry3Bb1 protein was DKC60-13 and its near isoline was DKC60-17. The transgenic hybrid expressing the Cry3Bb1 protein in 2006 was DKC60-18 (RR2/YGPL) and its isoline DKC60-19 (RR2/YGCB) (Monsanto Company). All hybrids in 2004 and 2006 were glyphosate resistant and had the same base genetics.

Rootworm Infestation and Egg Hatch. *Experiment 1.* In 2005, the western corn rootworm eggs were from the USDA-ARS NGIRL Brookings, SD diapausing colony. In 2006, wildtype eggs came from French Agricultural Research, Inc, Lamberton, MN. In both years, each row was infested with diapausing western corn rootworm eggs suspended in 0.15% agar solution at the rates of 2000 and 7025 eggs per m by using an egg infesting tractor described by Moellenbeck et al. (1994) modified after Sutter and Branson (1980). On 27 May 2005 and 17 May 2006 the eggs were infested (Table 1). The growth stage of the maize in 2005 and 2006 was V2-V3 and V1 respectively (Ritchie et al. 1992). The rootworm egg hatch was monitored by digging up single maize plants from an extra isoline row that was infested at the high egg density (Table 1). The presence of first instar larvae were confirmed by placing the root mass and surrounding soil into a tub containing around 8 liters of water, churning the root mass, and visually searching for the floating larvae.

Experiment 2. The 2004 and 2006 locations had a natural infestation of predominately western corn rootworm eggs. Egg hatch was monitored in both years by digging up extra maize plants around the anticipated egg hatch date and using Tullgren funnels to detect larvae. The first neonate larvae were found on 26 May 2004 and 30 May 2006 (Table 1).

Herbicide Application. *Experiment 1.* On 27 May 2005, the weed-free portions of each replication were sprayed with a pre-emergence application of atrazine / s-metachlor (Bicep II Magnum, Syngenta Corporation, Greensboro, NC) at the rate of 5.9 l / ha. From this point on the weed-free portions of the plot were rogued weekly and unwanted weeds were pulled from the foxtail portion within each replication. On 27 April 2006, the weed-free portion of each replication was sprayed with a pre-emergence application of s-metolaclof / atrazine / mesotrione (Lumax, Syngenta Corporation, Greensboro, NC) at a rate of 7.1 l / ha. On 26 May 2006 the foxtail portions of the plot were sprayed with 2,4-D Amine (0.59 l / ha) to control broadleaf weeds. The above herbicides were sprayed with a CO₂ backpack sprayer equipped with four 8001VS stainless steel spray tips and calibrated to deliver 140 liter/ha at 137.9 kPa(20 psi). In 2006 the weed-free portion of the study was spot sprayed as needed with a 2.5% solution of glyphosate (Touchdown Total™, Syngenta Corporation, Greensboro, NC) using a 8 liter Hudson hand sprayer (H. D. Hudson Manufacturing Company, Hasting, MN).

The foxtail portions of the studies were sprayed with a 2.5% solution of glyphosate using the Hudson hand sprayer on 17 June 2005 and 19 June 2006, which was four and six days, respectively, after initial egg hatch (Table 1). This was done to force the western corn rootworm larvae using the alternate host onto the maize roots. In 2005, the maize hybrids planted were not glyphosate resistant requiring the green foxtail to be first matted down between treatment rows so that it was not in physical contact with maize plants, then sprayed carefully using plywood boards to shield the maize from glyphosate drift. For the remainder of the 2005 field season the entire field plot was kept weed free through weekly roguing.

Experiment 2. On 17 May 2004 the zero weed density plots were sprayed with 3.5 l / ha of glyphosate + 3.08 l / ha of atrazine / s-metachlor + 1% AMS. On 28 April 2006, the weed-free portions of each replication were sprayed with s-metolachlor / atrazine / mesotrione at a rate of 7.1 l / ha. The CO₂ backpack sprayer described above was used to apply these herbicides. In both years the weed-free portion of the plots were spot sprayed as needed with 2.5% solution of glyphosate (Touchdown Total™, Syngenta Corporation, Greensboro, NC) using a Hudson hand sprayer. The whole plot was sprayed with 2,4-D Amine (0.59 l / ha) on 25 May 2006 to control broadleaf weeds. In 2004, the treatments with the 3, 7, and 14-day weed removal requirements were sprayed on May 28, June 4, and June 11 respectively (Table 1). In 2006, the treatments with the 3, 7, and 14 days weed removal requirements were sprayed on June 2, June 7, and June 14 respectively (Table 1). The treatments were sprayed with glyphosate (Touchdown Total™, Syngenta Corporation, Greensboro, NC) at a rate of 2.36 l / ha using the same CO₂ backpack sprayer as described above.

Beetle Sampling. In both experiments, the beetle emergence cages were centered over a live plant spanning mid-row-to-mid-row as modified after Hein and Tollefson (1985). Two cages were used per treatment for each replication. Each year traps were checked twice per week and were checked for at least 2 wk after the final adult was caught. All beetles caught were placed in individually labeled scintillation vials containing 95% ethanol and brought to the lab for processing. In both experiments, the number and sex of the beetles were recorded.

Experiment 1. There was one emergence trap per each treatment row with a total of 24 per replication and 96 total cages placed out. In 2005, the emergence cages were placed out between June 27 and July 1 and they were checked for adults between July 5 and August 22 (Table 1). In 2006, the emergence cages were placed out on June 20 and checked from June 29 through August 21 (Table 1). Initial beetle emergence was monitored in both years by watching other experiments at the same location, which were infested earlier.

Experiment 2. There was two emergence traps per treatment and a total of 180 for all five replications. In 2004, the cages were placed out on the 24 and 25 of June and checked between June 30 and August 16. In 2006, the emergence traps were placed out on June 12 and checked from June 19 until September 5. Initial adult emergence was monitored in both years by pulling early germinating volunteer maize plants and searching for western corn rootworm pupae.

Root Damage Evaluations. In both experiments, when $\approx 80\%$ of the larvae had, reached pupation, five randomly selected maize plants from each treatment were cut to a height of 0.6 m above ground. These plants were then dug up and the roots were washed and rated using the 0-3 node-injury scale (Oleson et al. 2005).

Experiment 1. In 2005, the maize roots were dug on July 19, washed on July 20 and 21, and rated on July 21. In 2006, the roots were dug, washed and rated on July 7 (Table 1).

Experiment 2. In 2004, the roots were dug on July 13, and washed and rated on July 14. In 2006, the roots were dug on June 26, and washed and rated on June 27 (Table 1).

Gene Checks. Once the emergence traps were placed in the field, all MON863 plants being examined for beetle emergence were assayed for expression of Cry3Bb1 protein using Lateral flow membrane strips (QuickStix™ Kit, Catalog number, AS 015 LS, EnviroLogix, Portland, ME) following the manufacturers protocol. When the roots were rated all MON863 maize plants with a rating of 0.5 or greater were also checked.

Statistical Analysis. *Experiment 1.* All data were analyzed as a factorial arrangement of a randomized complete block split-split-plot design using the PROC MIXED procedure of the statistical package SAS (SAS Institute 1990). The model contained the main plot of green foxtail density, the subplots of egg density and the sub sub-plots of insect management tactics. Beyond the standard analysis of variance (ANOVA), we preplanned a comparison of egg density and green foxtail density within each of the three insect management tactics and a comparison of insect management tactics within green foxtail density and egg density (within columns and rows of the tables). These were done with a least significant difference (LSD) for a split-split-plot design as described by Steele et al. (1997).

Experiment 2. All data were analyzed as a factorial arrangement of a randomized complete block design using the PROC MIXED procedure of the statistical package SAS (SAS Institute 1990). The model contained the factors of giant foxtail density, foxtail control times and the insect management tactics. Beyond the standard analysis of variance (ANOVA), we preplanned a comparison of insect management tactics and giant foxtail density within each of the three giant foxtail control times and a comparison foxtail control times within giant foxtail densities and insect management tactics (within columns and rows of the tables). These were done with a least significant difference

(LSD) for a factorial arrangement of a randomized complete block design as described by Steele et al. (1997).

Although untransformed data are shown in the tables for both experiments, to meet the assumptions of the analysis, all data were transformed by $\log(x + 1)$ before analysis. The $\log(x + 1)$ transformation was chosen to meet the assumptions of equal variance.

2.2 Results

Gene Checks. All the Bt maize plants that were selected tested positive and all isoline plants selected tested negative in the gene check test.

Beetle Emergence. *Experiment 1.* The total number of beetles that emerged in 2005 was significantly affected by egg density ($F = 5.91$; $df = 1, 18$; $P = 0.0258$), insect management tactics ($F = 17.83$; $df = 2, 12$; $P = 0.0003$), and the interaction of these two factors ($F = 3.86$; $df = 2, 18$; $P = 0.0402$). However, green foxtail density ($F = 0.70$; $df = 1, 3$; $P = 0.4645$) and the interaction of all three factors ($F = 0.22$; $df = 2, 18$; $P = 0.8067$) did not significantly affect beetle emergence. Significantly, more beetles emerged from isoline than MON863 for all treatments and main effects (Table 2). When comparing the weed-free versus weed plots, five out of the six comparisons had greater emergence in the weed-free plots but none of these differences were significant (Table 2). There was no significant difference though between male and female emergence for any treatment, though in all treatments, female beetle emergence was greater than or equal male emergence.

In 2006, significantly more beetles emerged from MON863 plus weeds than weed-free MON863 at the higher infestation level (Table 3). This was the first

documentation in the field that grassy weeds can significantly impact western corn rootworm survival on MON863. This difference was also significant for isoline maize at the high infestation level (Table 3). The total number of beetles that emerged was significantly affected by the insect management tactics ($F = 14.69$; $df = 2, 12$; $P = 0.0006$) and the three way interaction of insect management tactics \times green foxtail density \times egg density ($F = 4.22$; $df = 2, 18$; $P = 0.0315$). However, green foxtail density ($F = 4.54$; $df = 1, 3$; $P = 0.1230$), and egg density ($F = 1.86$; $df = 1, 18$; $P = 0.1897$) did not significantly affect beetle emergence. All 12 treatments had a higher number of female beetles emerging than male beetles. This difference was significant in one treatment with green foxtail and one treatment without green foxtail, which included the isoline-high and the isoline-low (insect management tactic-egg density), respectively (data not shown).

Experiment 2. The total number of beetles that emerged in 2004 was significantly affected by insect management tactics ($F = 66.34$; $df = 1, 68$; $P < 0.0001$). However, giant foxtail densities ($F = 0.30$; $df = 2, 68$; $P = 0.7454$), foxtail control times ($F = 0.21$; $df = 2, 68$; $P = 0.8106$) and the interaction among all three factors ($F = 0.18$; $df = 4, 68$; $P = 0.9502$) did not significantly affect beetle emergence. Overall, beetle emergence in 2004 was low compared to the 2006 season. All isoline treatments produced significantly more beetles than the transgenic treatments (Table 4). No significant difference was found between weed-free plots and weedy plots for any treatment (Table 4). More females than males were recovered for all isoline and transgenic treatments. Five out of the nine isoline treatments had a significantly greater female beetle emergence which

included the none-late, med-mid, med-early, high-late, and high-mid treatments (giant foxtail density-control time) (Data not shown).

In 2006, beetle emergence was affected by insect management tactics ($F = 12.07$; $df = 1, 68$; $P = 0.0009$), giant foxtail density ($F = 3.36$; $df = 2, 68$; $P = 0.0405$), and the three way interaction of insect management tactics \times giant foxtail density \times giant foxtail control times ($F = 2.71$, $df = 4, 68$; $P = 0.0374$). However, the giant foxtail control times ($F = 0.83$; $df = 2, 68$; $P = 0.4408$) did not significantly affect the beetle emergence.

Within the MON863 treatments, significantly more beetles emerged from the weed-free plots than the high density plots of giant foxtail that was sprayed 3d after mean egg hatch (Table 5). Overall, the number that emerged from MON863 was not significantly different than the number that emerged from isoline maize for 7 of 9 comparisons when weed density and control time were the same in isoline and MON863. There was no significant difference in the number of males and females that emerged in the isoline treatments, but the number of males was higher in five of the nine treatments. All the transgenic treatments had a higher number of females that emerged but only the zero giant foxtail density at the late control measure (14 d) was significant. Overall, adult emergence was greater in 2006 than in 2004 and started 11 days earlier and lasted 20 days later than the 2004 experiment.

Root Damage. *Experiment 1.* In 2005, root damage was significantly affected by green foxtail density ($F = 21.25$; $df = 1, 3$; $P = 0.0192$), egg density ($F = 4.73$; $df = 1, 18$; $P = 0.0433$), insect management tactics ($F = 253.72$, $df = 2, 12$; $P < 0.0001$) and the interaction of insect management tactics \times green foxtail density ($F = 3.93$; $df = 2, 12$; $P =$

0.0487). The three way interaction ($F = 0.27$; $df = 2, 18$; $P = 0.7667$) did not significantly affect the root damage. Overall, the weed-free treatments had higher root damage ratings than the treatments with the heavy green foxtail density (Table 6). In comparison of the similar treatments with and without green foxtail there was a significant difference in root damage in the isoline-high, isoline + tefluthrin-high, and the isoline-low (insect management tactic-egg density) treatments. There was a significant difference between all three insect management tactic main effects at both egg densities.

In 2006, root damage was significantly affected by egg density ($F = 26.35$; $df = 1, 18$; $P < 0.0001$), insect management tactics ($F = 60.31$; $df = 2, 12$; $P < 0.0001$) and interaction of these two factors ($F = 19.13$; $df = 2, 18$; $P < 0.0001$). However, green foxtail density ($F = 0.09$; $df = 1, 3$; $P = 0.7886$) and the interaction of all three factors ($F = 0.32$; $df = 2, 18$; $P = 0.7289$) did not significantly affect root damage. The three treatments with the moderate green foxtail density (9.1 plants / m²) at the high egg density (7025 eggs / m) along with the transgenic hybrid at the low egg density had a higher but not significant root rating than the similar weed free treatments (Table 7). The other two treatments (isoline and isoline + tefluthrin) at the low egg density had higher but not significant root ratings in the weed free treatments. There was a significant difference between all the insect management tactic main effects at the high egg density, but only a significant difference between the two-isoline insect management tactic main effects and the transgenic main effect at the low egg density.

Experiment 2. In 2004, root damage was significantly affected by insect management tactics ($F = 165.50$; $df = 1, 67$; $P < 0.0001$) and giant foxtail density ($F = 3.40$; $df = 2, 67$;

$P = 0.0393$). However, giant foxtail control times ($F = 1.50$; $df = 2, 67$; $P = 0.2298$) and the interaction of all three factors ($F = 0.31$; $df = 4, 67$; $P = 0.8686$) did not significantly affect root damage. The root damage ratings on all isoline hybrid treatments were significantly greater than the root damage on the transgenic hybrid treatments (Table 8). The main effect of giant foxtail density showed maximum root damage at the 9.1 plant density for both the transgenic and isoline hybrids but only a significant difference in the isoline hybrid. The main effect of giant foxtail control time for the isoline hybrid showed significantly greater damage at the early and late control times compared to the middle control time.

In 2006, root damage was significantly affected by insect management tactics ($F = 326.00$; $df = 1, 68$; $P < 0.0001$). However, giant foxtail density ($F = 2.26$; $df = 2, 68$; $P = 0.1122$), giant foxtail control times ($F = 0.01$; $df = 2, 68$; $P = 0.9919$), and the interaction of all three factors ($F = 0.84$; $df = 4, 68$; $P = 0.5045$) did not significantly affect root damage. The root damage ratings on all isoline hybrid treatments were significantly greater than the damage on the transgenic hybrid treatments (Table 9). The main effect of giant foxtail density showed maximum root damage at the 23.7 and the 9.1 plant density for the isoline and transgenic hybrids, respectively, but there were only significant differences within the isoline main effects.

2.3 Discussion

In both experiments and all three years, the transgenic maize hybrid expressing the Cry3Bb1 protein had significantly less root damage than its near isoline hybrid. Insect management tactics significantly affected beetle emergence and root damage in

both experiments. Overall, female beetle emergence was greater than or equal to male emergence in all the transgenic treatments.

Experiment 1. Beetle emergence was very different between 2005 and 2006, and the effect of weeds had opposite effects in the two years. In 2005, green foxtail density was extremely high (1017/m²). In 2006, green foxtail density was thinned to a level that may be more typical of what might be found in a slightly weedy commercial field (~9/m²). Green foxtail density may partially explain the differences in the effects of foxtail between the two years. In 2005, the heavy green foxtail density led to less root biomass in the maize plants with weeds versus without weeds (personal observation). In 2006, there was not a pronounced difference between the size of maize roots with and without weeds. Since maize is a superior host, the size of the maize roots could be a mechanism for the differential effects of weeds between the two years. The reduced beetle emergence in a heavy foxtail density is in agreement with Johnson et al. (1984).

When comparing the transgenic hybrid to the isoline hybrid, beetle emergence from the transgenic hybrid was delayed and drawn out four to ten days longer depending on the year. In 2006, four of the treatments with the moderate green foxtail density had higher root damage ratings than their similar weed-free treatments and this subsequently led to greater beetle emergence in three of those four treatments. Overall, beetle emergence in 2006 was greater in four out of the six foxtail plots than the corresponding weed-free plot. In fact, for transgenic treatments at the high egg density more than five fold the number of beetles emerged from transgenic maize plus weeds than weed-free transgenic maize, a significant difference, supporting the conclusions of Oyediran et al. (2005) for at least some field situations.

Experiment 2. The giant foxtail density significantly affected root damage in both years and adult emergence in 2006. In both years, the treatment means for the isoline hybrids had significantly higher root damage than the treatment means for the transgenic hybrid. However, the giant foxtail control time did not significantly affect either maize damage ratings or western corn rootworm beetle emergence in either year. In both years, all but one isoline treatment produced more beetles than their similar transgenic treatments. However, this difference between the similar isoline and transgenic treatments was not significant in five out of six weed treatments in 2006 (Table 5). In 2004 and 2006, the highest beetle emergence means were both achieved at the medium weed density. In 2006, adult emergence for the zero foxtail density main effect for the transgenic hybrid was greater than the mean adult emergence from transgenic maize for the six giant foxtail treatment means (Table 5).

Conclusions. Part of the goal of the current experiment was to see if the presence of grassy weeds would serve as a bridge to increased survival from and or increased damage to transgenic maize. For Experiment 1 and 2, there was no significant difference in plant damage between transgenic maize with and without weeds (Tables 6-9). In addition, in 16 possible comparisons between adult emergence from transgenic maize with and without weeds, adult emergence was significantly higher from transgenic maize with weeds in only one of these comparisons (Tables 2-5). In that comparison (Table 3, the high egg density), more than 5 fold the number of beetles were produced from transgenic maize with weeds than without weeds. Apparently, weeds have little effect on survival on transgenic maize under many circumstances, but can under certain situations. In

general, only a slight trend towards increased damage and beetle emergence can be seen with transgenic maize + foxtail. Without knowing the impact of beetles that are produced from larvae that fed on foxtail and transgenic maize roots have on resistance management in most situations, we believe a conservative approach should be taken. This approach would include control of grassy weeds before WCR egg hatch, which seems to be around late May in central Missouri in a typical year. If there is, a trend toward the use of transgenic hybrids stacked with the rootworm and glyphosate resistance, the control of weeds may not occur in a timely way, assuming that the producer uses only glyphosate to control his weeds. Factors that may contribute to this is weather and the fact that producers may be holding off on the last glyphosate application till shortly before the maize plants canopy the row. One possible solution to this problem would be to use a pre-emerge grass herbicide. This option would support the resistant management of weeds to glyphosate by adding another mode of action to weed control. It would also allow the producer to come back with one timely application of glyphosate to control the remaining weeds especially large seeded weeds like sunflower (*Helianthus spp.*), cocklebur (*Xanthium strumarium*), and shattercane (*Sorghum bicolor*) that are hard to control with a pre-emergence herbicide. This option would also keep grass pressure to a minimum at egg hatch and reduce the risk of increased damage and higher numbers of beetles being produced. The impact of this plan on WCR resistance management would be unknown though, because it is not know whether the partial development on an alternate host or a non-Bt plant is positive or negative to the long-term resistance management.

Table 1. Important field activity dates for Experiment 1 and Experiment 2

Field Activities	Experiment 1		Experiment 2	
	2005	2006	2004	2006
Foxtail planting	20-Apr	17-Apr	n/a	21-Apr
Maize Planting	3-May	25-Apr	7-May	21-Apr
WCR egg infestation	27-May	17-May	n/a	n/a
Egg hatch	13-Jun	13-Jun	26-May	30-May
Glyphosate spray date 1	17-Jun	19-Jun	28-May	2-Jun
Glyphosate spray date 2	n/a	n/a	4-Jun	7-Jun
Glyphosate spray date 3	n/a	n/a	11-Jun	14-Jun
Root rating	21-Jul	7-Jul	21-Jul	27-Jun
Beetle sampling	5-Jul → 22-Aug	29-Jun → 21-Aug	30-Jun → 16-Aug	19-Jun → 5-Sep

n/a, not applicable.

Table 2. Mean \pm SE number of western corn rootworm that emerged per plot in 2005 (Experiment 1)

Egg density (eggs / m)	<i>S. viridis</i> (plants / m ²)	Insect management tactic		
		Isoline	Isoline + tefluthrin	Transgenic
7025	9.1	15.0 \pm 5.9ABa	5.8 \pm 4.2ABab	0.5 \pm 0.3Ab
7025	0	31.0 \pm 9.6Aa	6.8 \pm 4.0Ab	1.0 \pm 0.7Ab
2000	9.1	5.8 \pm 3.0Ca	4.3 \pm 1.5BCa	0.3 \pm 0.3Ab
2000	0	8.0 \pm 4.9BCa	4.0 \pm 2.0Cab	0.5 \pm 0.3Ab
Insect management main effect at 7025 eggs / m		23.0 \pm 8.0a	6.3 \pm 0.5b	0.8 \pm 0.3c
Insect management main effect at 2000 eggs / m		6.9 \pm 1.1a	4.1 \pm 0.1a	0.4 \pm 0.1b

Significant differences ($P = 0.05$) between egg density and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management tactics within a row are indicated by different lowercase letters. Although untransformed data are shown, data were analyzed as $\log(x + 1)$.

Table 3. Mean ± SE number of western corn rootworm that emerged per plot in 2006 (Experiment 1)

Egg density (eggs / m)	<i>S. viridis</i> (plants / m ²)	Insect management tactic		
		Isoline	Isoline + tefluthrin	Transgenic
7025	9.1	83.0 ± 30.3Aa	18.3 ± 9.7Ab	20.5 ± 8.7Ab
7025	0	28.5 ± 14.7Ba	26.3 ± 6.5Aa	3.8 ± 1.4Bb
2000	9.1	32.5 ± 15.9ABa	26.5 ± 4.3Aa	5.8 ± 1.4ABb
2000	0	38.0 ± 10.6ABa	9.8 ± 2.1Ab	3.5 ± 1.6Bb
Insect management main effect at 7025 eggs / m		49.8 ± 27.3a	22.3 ± 4.0ab	12.1 ± 8.4b
Insect management main effect at 2000 eggs / m		35.3 ± 2.8a	18.1 ± 8.4a	4.6 ± 1.1b

Significant differences ($P = 0.05$) between egg density and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management tactics within a row are indicated by different lowercase letters. Although untransformed data are shown, data were analyzed as $\log(x + 1)$.

Table 4. Mean \pm SE number of western corn rootworm that emerged per plot in 2004 (Experiment 2)

Insect management tactic	<i>S. faberi</i> density (plants / m ²)	<i>S. faberi</i> control time (d after mean hatch)			<i>S. faberi</i> density main effect
		3	7	14	
Isoline	0	6.4 \pm 3.1Aa	3.4 \pm 1.2Aa	8.2 \pm 3.4Aa	6.0 \pm 1.4A
	9.1	7.8 \pm 2.5Aa	9.6 \pm 3.8Aa	5.6 \pm 1.0Aa	7.7 \pm 1.2A
	23.7	6.4 \pm 1.9Aa	7.2 \pm 3.4Aa	8.8 \pm 5.4Aa	7.5 \pm 0.7A
Transgenic	0	1.0 \pm 0.6Ba	1.2 \pm 0.6Ba	1.8 \pm 0.8Ba	1.3 \pm 0.2B
	9.1	0.8 \pm 0.6Ba	1.4 \pm 0.6Ba	0.6 \pm 0.3Ba	0.9 \pm 0.2B
	23.7	1.0 \pm 0.6Ba	0.4 \pm 0.3Ba	0.8 \pm 0.4Ba	0.7 \pm 0.2B
Control time main effect (Isoline)		6.9 \pm 0.5a	6.7 \pm 1.8a	7.5 \pm 1.0a	
Control time main effect (Transgenic)		0.9 \pm 0.1a	1.0 \pm 0.3a	1.1 \pm 0.4a	

Significant differences ($P = 0.05$) between insect management tactics and giant foxtail density combinations within a column are indicated by different uppercase letters. Significant differences between giant foxtail control times within a row are indicated by different lowercase letters. Although untransformed data are shown, data were analyzed as $\log(x + 1)$.

Table 5. Mean \pm SE number of western corn rootworm that emerged per plot in 2006 (Experiment 2)

Insect management tactic	<i>S. faberi</i> density (plants / m ²)	<i>S. faberi</i> control time (d after mean hatch)			<i>S. faberi</i> density main effect
		3	7	14	
Isoline	0	23.2 \pm 6.7ABa	38.0 \pm 7.5Aa	41.6 \pm 19.7Aa	34.3 \pm 5.6A
	9.1	40.0 \pm 10.8Aa	24.4 \pm 4.9ABab	13.6 \pm 4.7ABb	26.0 \pm 7.7A
	23.7	26.2 \pm 7.3Aa	14.0 \pm 4.5Ba	19.0 \pm 6.0ABa	19.7 \pm 3.5AB
Transgenic	0	25.6 \pm 4.4Aa	9.8 \pm 2.2Ba	19.8 \pm 5.0ABa	18.4 \pm 4.6ABC
	9.1	16.4 \pm 5.8ABa	10.4 \pm 1.0Ba	13.2 \pm 3.6Ba	13.3 \pm 1.7BC
	23.7	10.0 \pm 4.3Ba	12.6 \pm 6.2Ba	10.8 \pm 3.7Ba	11.1 \pm 0.8C
Control time main effect (Isoline)		29.8 \pm 5.2a	25.5 \pm 6.9a	24.7 \pm 8.6a	
Control time main effect (Transgenic)		17.3 \pm 4.5a	10.9 \pm 0.9a	14.6 \pm 2.7a	

Significant differences ($P = 0.05$) between insect management tactics and giant foxtail density combinations within a column are indicated by different uppercase letters. Significant differences between giant foxtail control times within a row are indicated by different lowercase letters. Although untransformed data are shown, data were analyzed as $\log(x + 1)$.

Table 6. Mean \pm SE root damage ratings for 2005 (Experiment 1)

Egg density (eggs / m)	<i>S. viridis</i> (plants / m ²)	Insect management tactic		
		Isoline	Isoline + tefluthrin	Transgenic
7025	9.1	1.09 \pm 0.15BCa	0.68 \pm 0.06Bb	0.02 \pm 0.02Ac
7025	0	1.69 \pm 0.10Aa	1.04 \pm 0.10Ab	0.04 \pm 0.01Ac
2000	9.1	0.98 \pm 0.09Ca	0.56 \pm 0.08Bb	0.04 \pm 0.01Ac
2000	0	1.33 \pm 0.04ABa	0.79 \pm 0.17ABb	0.07 \pm 0.03Ac
Insect management main effect at 7025 eggs / m		1.39 \pm 0.30a	0.86 \pm 0.18b	0.03 \pm 0.01c
Insect management main effect at 2000 eggs / m		1.15 \pm 0.17a	0.67 \pm 0.12b	0.05 \pm 0.01c

Significant differences ($P = 0.05$) between egg density and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management tactics within a row are indicated by different lowercase letters. Although untransformed data are shown, data were analyzed as $\log(x + 1)$.

Table 7. Mean \pm SE root damage ratings for 2006 (Experiment 1)

Egg density (eggs / m)	<i>S. viridis</i> (plants / m ²)	Insect management tactic		
		Isoline	Isoline + tefluthrin	Transgenic
7025	9.1	1.41 \pm 0.28Aa	0.65 \pm 0.26Ab	0.04 \pm 0.01Ac
7025	0	1.20 \pm 0.28Aa	0.57 \pm 0.26Ab	0.02 \pm 0.006Ac
2000	9.1	0.40 \pm 0.16Ba	0.44 \pm 0.12Aa	0.05 \pm 0.02Ab
2000	0	0.45 \pm 0.11Ba	0.54 \pm 0.16Aa	0.04 \pm 0.02Ab
Insect management main effect at 7025 eggs / m		1.30 \pm 0.11a	0.61 \pm 0.04b	0.03 \pm 0.01c
Insect management main effect at 2000 eggs / m		0.42 \pm 0.02a	0.49 \pm 0.05a	0.04 \pm 0.004b

Significant differences ($P = 0.05$) between egg density and management combinations within a column are indicated by different uppercase letters. Significant differences between insect management tactics within a row are indicated by different lowercase letters. Although untransformed data are shown, data were analyzed as $\log(x + 1)$.

Table 8. Mean \pm SE root damage ratings for 2004 (Experiment 2)

Insect management tactic	<i>S. faberi</i> density (plants / m ²)	<i>S. faberi</i> control time (d after mean hatch)			<i>S. faberi</i> density main effect
		3	7	14	
Isoline	0	1.27 \pm 0.39Aa	0.98 \pm 0.29Aa	0.91 \pm 0.27Ba	1.06 \pm 0.11B
	9.1	1.77 \pm 0.16Aa	1.41 \pm 0.47Aa	1.86 \pm 0.34Aa	1.68 \pm 0.14A
	23.7	1.25 \pm 0.26Aab	0.75 \pm 0.29Ab	1.56 \pm 0.42ABa	1.19 \pm 0.24B
Transgenic	0	0.08 \pm 0.02Ba	0.09 \pm 0.01Ba	0.07 \pm 0.01Ca	0.08 \pm 0.01C
	9.1	0.09 \pm 0.01Ba	0.14 \pm 0.04Ba	0.16 \pm 0.05Ca	0.13 \pm 0.01C
	23.7	0.13 \pm 0.03Ba	0.08 \pm 0.02Ba	0.11 \pm 0.02Ca	0.10 \pm 0.01C
Control time main effect (Isoline)		1.43 \pm 0.17a	1.05 \pm 0.19b	1.44 \pm 0.28a	
Control time main effect (Transgenic)		0.11 \pm 0.01a	0.10 \pm 0.02a	0.11 \pm 0.03a	

Significant differences ($P = 0.05$) between insect management tactics and giant foxtail density combinations within a column are indicated by different uppercase letters. Significant differences between giant foxtail control times within a row are indicated by different lowercase letters. Although untransformed data are shown, data were analyzed as $\log(x + 1)$.

Table 9. Mean \pm SE root damage ratings for 2006 (Experiment 2)

Insect management tactic	<i>S. faberi</i> density (plants / m ²)	<i>S. faberi</i> control time (d after mean hatch)			<i>S. faberi</i> density main effect
		3	7	14	
Isoline	0	1.05 \pm 0.19Aa	1.30 \pm 0.16Aa	0.84 \pm 0.08Ba	1.06 \pm 0.13B
	9.1	1.36 \pm 0.35Aa	1.15 \pm 0.24Aa	1.38 \pm 0.39ABa	1.30 \pm 0.07AB
	23.7	1.35 \pm 0.20Aa	1.55 \pm 0.31Ab	1.80 \pm 0.27Aa	1.50 \pm 0.13A
Transgenic	0	0.08 \pm 0.03Ba	0.01 \pm 0.002Ba	0.07 \pm 0.03Ca	0.05 \pm 0.02C
	9.1	0.09 \pm 0.03Ba	0.06 \pm 0.03Ba	0.05 \pm 0.01Ca	0.07 \pm 0.01C
	23.7	0.07 \pm 0.05Ba	0.07 \pm 0.04Ba	0.04 \pm 0.03Ca	0.06 \pm 0.01C
Control time main effect (Isoline)		1.25 \pm 0.10a	1.02 \pm 0.12a	1.34 \pm 0.28a	
Control time main effect (Transgenic)		0.08 \pm 0.01a	0.05 \pm 0.02a	0.06 \pm 0.01a	

Significant differences ($P = 0.05$) between insect management tactics and giant foxtail density combinations within a column are indicated by different uppercase letters. Significant differences between giant foxtail control times within a row are indicated by different lowercase letters. Although untransformed data are shown, data were analyzed as $\log(x + 1)$.

CHAPTER 3

Introduction

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, (Chrysomelidae, Coleoptera), is a pest in continuous maize (*Zea mays* L.) fields and in some first year maize fields. It is primarily a pest in its larval stages where it feeds almost exclusively on maize roots (Branson and Ortman 1970). The root damage can disrupt water relations (Riedell 1990), reduce nutrient uptake (Kahler et al. 1985), cause reductions in photosynthetic rate (Godfrey et al. 1993), and reduce plant stability (Spike and Tollefson 1989). In the adult stage, western corn rootworm beetles will feed on silks, maize pollen, and developing kernels on the ears (Gray and Tollefson 1988; Spike and Tollefson 1989). Unlike the larval stage, western corn rootworm beetles do not usually cause much damage or yield loss. Under extremely high populations, they can cause pollination problems.

The nutritional ecology of western corn rootworm larvae is relatively poorly understood for such a major pest. It is known that western corn rootworm larvae can develop on a number of grass species (Branson and Ortman 1967, 1970, Clark and Hibbard 2004, Wilson and Hibbard 2004, Oyediran et al. 2004). It is also known that plant phenology can effect development of western corn rootworm larvae on alternate hosts (Chege et al. 2005) and maize (Stavitski and Davis 1997), where late hatching larvae did poorly on early maturing maize. Why western corn rootworm larvae can survive and develop on some grass species, but not others is not known, nor is it known what aspects of older roots make them less suitable for western corn rootworm larval development.

In an elegant set of experiments, Moeser and Vidal (2004a, b) measured weight gain (or loss) of second instar western corn rootworm larvae and the amount of food consumed by the same individual larva to calculate food conversion indexes for a series of alternate hosts and maize varieties respectively. In addition, they evaluated the carbon/nitrogen ratio and the phytosterol content of the alternate hosts and maize varieties. For alternate hosts, the plant species with higher nitrogen content were less suitable for western corn rootworm development. Phytosterol content was positively correlated with the amount of food consumed, but not the weight gain of the insects. For maize, nitrogen content was positively correlated with converting root biomass into insect biomass and phytosterol content influenced larval weight gain and the amount of infested food. The change in weight of the second instar larvae evaluated ranged from strongly positive to strongly negative values for both alternate hosts and maize varieties. Unfortunately, in both studies any differences in weight gain (or loss) of the larvae and the food conversion indices they calculated are confounded with unknown differences in the speed that a plant species or maize variety senesces, since the same severed roots were the only food source for six days.

The effects of senescing root has been evaluated to some extent in the field previously. One way to capture adults as they emerge from the soil is to sever the plant below the growing point (which kills maize plants) and to place an emergence cage over the plant (Krysan and Miller 1986). Fisher (1984) evaluated adult emergence from above maize plants that had been severed below the growing point and compared these data to adult emergence from living maize plants in the same field. He found that although severing the plants accelerated the time frame for adult emergence, the total number of

adults emerged was not significantly different between the two treatments when the timing of severing was initiated at the predominant pupae stage. Based upon adult emergence data from maize plants severed below the growing point, it was definitely reasonable for Moeser and Vidal (2004a, b) to assume that cut roots may serve as suitable hosts. Riedell and Kim (1990) also evaluated cut roots of maize when evaluating the tissue types that western corn rootworm larvae feed on. They found that western corn rootworm larvae primarily fed on the cortex tissue.

The importance of the timing that senescing roots of alternate hosts killed with herbicide become unsuitable for larval nutrition has just recently become important to consider. Oyediran et al. (2005) demonstrated that significantly more western corn rootworm adults emerged from transgenic, rootworm-resistant maize (Cry3Bb1-expressing) when combined with grassy weeds that were sprayed with glyphosate 4 d after infestation of neonate larvae than emerged from Cry3Bb1-expressing maize kept weed free or a living grassy weed control. Oyediran et al. (2005) assumed that neonate western corn rootworm larvae initially established on the grassy weeds and whenever the weeds became unsuitable due to the herbicide spray, the larger larvae were able to better survive the Cry3Bb1-expressing maize. The goal of the current paper was to determine the timing that senescing root tissues of *Setaria faberi* R.A.W. Herrm. and maize become unsuitable for growth of neonate and second instar western corn rootworm larvae.

3.1 Materials and Methods

There were three separate experiments, which were quite similar in principle, but were conducted and analyzed separately. In the first experiment, glyphosate was used to kill *S. faberi*. In the second experiment, glyphosate was used to kill maize. In the final

experiment, maize was killed by severing it below the growing point. These experiments evaluated western corn rootworm larvae for survival and growth parameters among the 12 to 14 treatments (Table 1). Each experiment had two trials, with five replications per trial. The main plot was the 12 to 14 treatments arranged in a randomized complete block split in space design. The subplot consisted of four pots per treatment and 12 to 14 treatments per replication that were randomly assigned a sample date for larval recovery (5, 10, and 15 day after infestation) or adult emergence. The plants used were maize (DKC 60-15, Monsanto Company, St. Louis, MO) and *Setaria faberi* (Azlin Seed Service, Leland, MS).

Plant Growing Conditions. Seeds were planted in pots containing 2:1 mixture of autoclaved soil/peat-based growing medium (Promix, Premier Horticulture LTEÉ, Quebec, Canada). Clay 3.8-liter pots were used for larval recovery, plastic 19-liter pots were used for adult emergence in the maize experiments, and 3.8-liter clay pots were used for the whole *S. faberi* experiment. Drainage holes in the pots were covered with a fine (114 μm per opening) stainless steel mesh (TWP Inc., Berkley, CA) to prevent larval escapes (Clark and Hibbard 2004). A photoperiod of 14:10 (L:D) h was maintained with 1000-W sodium bulbs (GE Lighting, Cleveland, OH). All plants were watered as needed and fertilized (Scotts peters Professional 20-20-20, Scotts-Seirra Horticultural Products Corp., Marysville, OH) every 7 d.

Larval Preparations. The nondiapausing western corn rootworm larvae used for these experiments were obtained from a colony maintained in our laboratory and was subsidized as needed by eggs from the USDA-ARS Northern Grain Insects Research Laboratory (NGIRL) in Brookings, SD and French Agricultural Research, Lamberton,

MN. After recovery from oviposition dishes, western corn rootworm eggs were placed in 708 ml Gladware[®] semi-durable plastic containers (Clorox, Australia Pty Limited 2005) with a thin layer of soil (same as above) for about 12 days at 25°C before the neonate larvae were needed. Neonates were infested within 24 hrs after they hatched. Second instars were obtained by infesting 125 neonate larvae on maize plants (≈15 g of untreated DKC 60-15 maize seed) that were planted in 708 ml Gladware[®] semi-durable plastic containers (Clorox, Australia Pty Limited 2005) in a growth chamber at 25°C five days earlier. Larvae were allowed to feed on these maize plants for six to eight days (in one instance up to 11 d) before larvae were recovered by the use of Tullgren funnels. Second instars were infested within a few hours after they were first placed into the funnels. Both neonates and second instars were infested with camel hairbrushes and the larvae were placed gently at the base of the maize or *Setaria faberi* plants. With each experiment, 100 larvae were randomly sampled from each colony for that experiment for both neonates and second instars and transferred to labeled glass scintillation vials containing 95% ethanol. If second instars of different ages were used for certain replications, these too were sampled and the replicate that they were used for was recorded. These larvae were used to find the average dry weight and head capsule width of larvae that began each experiment.

Glyphosate Sprayed *S. faberi*.

Trial 1. One gram of *S. faberi* seed was planted per pot on February 3 (Treatments 11 and 12), February 8 (Treatments 9 and 10), and February 13 (Treatments 1-8). Maize controls (4 seeds) were planted on February 8. Emerged maize control plants were

thinned down to two plants / pot on February 21. *S. faberi* plants were sprayed with a 2.4% solution of glyphosate on March 3 (Treatments 7, 8, 11, and 12), March 8 (Treatments 5, 6, 9, and 10), and March 13 (Treatments 3 and 4). *S. faberi* plants grew 20 to 30 cm in height depending on the treatment. A HOBO recorder (model H08-001-02, Bourne, MA) recorded hourly temperatures from February 3 thru May 13 with an average reading of $26 \pm 0.09^{\circ}\text{C}$ (Min 14°C Max 49°C).

The odd numbered treatments were infested with 30 neonate larvae and the even numbered treatments were infested with eight (rep 1, 4, and 5), seven (rep 3), and five (rep 2) second instars. All second instar larvae were infested on March 13. Neonates were infested on March 13 (Reps 1-3) and March 14 (Reps 4-5). Larval recovery dates were March 18 and 19 (5 d), March 23 and 24 (10 d), and March 28 and 29 (15 d).

Trial 2. One gram of *S. faberi* seed was planted per pot on May 26 (Treatments 11 and 12), May 31 (Treatments 9 and 10), and June 5 (Treatments 1-8). Maize controls (4 seeds) were planted on May 31. Emerged maize control plants were thinned down to two plants / pot on June 12. *S. faberi* plants were sprayed with a 2.4% solution of Touchdown TotalTM (Syngenta) on June 23 (Treatments 7, 8, 11, and 12), June 28 (Treatments 5, 6, 9, and 10), and July 3 (Treatments 3 and 4). *S. faberi* plants grew 30 to 40cm in height depending on the treatment. A HOBO recorder (model H08-001-02, Bourne, MA) recorded hourly temperatures from May 26 thru August 23 with an average reading of $27 \pm 0.06^{\circ}\text{C}$ (Min 21°C Max 37°C).

The odd numbered treatments were infested with 30 neonate larvae and the even numbered treatments were infested with 8-second instars. All larvae were infested on July 3. Larval recovery dates were July 8 (5 d), July 13 (10 d), and July 18 (15 d).

Severed Maize Experiment.

Trial 1. Three maize seeds were planted per pot on January 6 (Treatments 11 and 12), January 11 (Treatments 9 and 10), and January 16 (Treatments 1-8). Emerged plants were thinned down to one plant/ pot on January 20 and 27. Maize plants were severed below the growing point on February 17 (Treatments 7, 8, 11, and 12), February 22 (Treatments 5, 6, 9, and 10), and February 27 (Treatments 3 and 4). The maize growth stages varied from V5 to V7 depending on the treatment. As with other experiments, a HOBO recorder recorded hourly temperatures from January 6 thru April 24 with an average reading of $23 \pm 0.06^{\circ}\text{C}$ (Min 13°C Max 34°C).

The odd numbered treatments were infested with 30 neonate larvae and the even numbered treatments were infested with 10-second instars. All second instar larvae were infested on February 27. Neonates were infested on February 26 (Reps 1-2) and February 27 (Reps 3-5). Larval recovery dates were February 3 and 4 (5 d), February 8 and 9 (10 d), and February 13 and 14 (15 d).

Trial 2. Maize was planted on Feb 17 (Treatments 11 and 12), Feb 22 (Treatments 9 and 10), and Feb 27 (Treatments 1-8). Emerged plants were thinned down to one plant/ pot on March 5. Maize plants were severed below the growing point on March 31 (Treatments 7, 8, 11, and 12), April 5 (Treatments 5, 6, 9, and 10), and April 10 (Treatments 3 and 4). The maize growth stages varied from V6 to V8 depending on the treatment. Recorded hourly temperatures from March 7 thru June 9 had an average reading of $25 \pm 0.11^{\circ}\text{C}$ (Min 11°C Max 42°C).

The odd numbered treatments were infested with 30 neonate larvae and the even numbered treatments were infested with 10-second instars. All neonate and second instar larvae were infested on April 10 for the larval recovery (clay) pots. Second instars were infested on April 10 for the adult emergence and neonates were infested on April 11. Larval recovery dates were April 15 (5 d), April 20 (10 d), and April 25 (15 d).

Glyphosate Sprayed Maize Experiment.

Trial 1. Three maize seeds were planted per pot on Feb 17 (Treatments 11 and 12), Feb 22 (Treatments 9 and 10), and Feb 27 (Treatments 1-8). Emerged plants were thinned to one plant/ pot on March 5. Maize plants were sprayed with a 2.4% solution of Touchdown Total™ (Syngenta) on March 31 (Treatments 7, 8, 11, and 12), April 5 (Treatments 5, 6, 9, and 10), and April 10 (Treatments 3 and 4). The maize growth stages varied from V6 to V8 depending on the treatment. A HOBO recorder (model H08-001-02, Bourne, MA) recorded hourly temperatures from March 7 thru June 9 with an average reading of 25 ± 0.11 SE °C (Min 11°C Max 42°C).

The odd numbered treatments were infested with 30 neonate larvae and the even numbered treatments were infested with 10 second instars. All neonate and second instars larvae were infested on April 10 for the larval recovery (clay) pots. Second instars were infested on April 10 for the adult emergence and neonates were infested on April 11. Larval recovery dates were April 15 (5 d), April 20 (10 d), and April 25 (15 d).

Trial 2. Maize was planted on May 26 (Treatments 11 and 12), May 31 (Treatments 9 and 10), and June 5 (Treatments 1-8). Emerged plants were thinned down to one plant/ pot on June 12. Maize plants were sprayed with a 2.4% solution of Touchdown Total™

(Syngenta) on July 7 (Treatments 7, 8, 11, and 12), July 12 (Treatments 5, 6, 9, and 10), and July 17 (Treatments 3 and 4). The maize growth stages varied from V7 to V11 depending on the treatment. A HOBO recorder (model H08-001-02, Bourne, MA) recorded hourly temperatures from July 24 thru August 28 with an average reading of $28 \pm 0.20^{\circ}\text{C}$ (Min 18°C Max 42°C).

The odd numbered treatments were infested with 30 neonate larvae and the even numbered treatments were infested with 10-second instars. All neonate and second instar larvae were infested on July 17. Larval recovery dates were July 22 (5 d), July 27 (10 d), and August 1 (15 d).

Larval Recovery. At 5, 10, and 15 days after infestation the contents of each pot (soil mixture, roots, and larvae) were individually placed in Tullgren funnels equipped with a 60 W light bulb (Great value, Soft White, Wal-Mart Company) for the extraction of the larvae. Collection jars containing water were placed under each funnel and checked 2 and 4 d later for larvae. Larvae were visually identified and removed using a camel hair paintbrush. Larvae recovered were counted and transferred to individually labeled scintillation vials containing 95% ethanol. The head capsule width (HCW) of each larvae were measured using an ocular micrometer (10x/21, Wild Co., Heerbrugg, Switzerland) mounted on a microscope (M3Z, Wild Co., Heerbrugg, Switzerland). Dry weights of the larvae were determined using an analytical scale (ER-182A, A and D Co., Tokyo, Japan) after placing the larvae in a desiccating oven (Thelco model 16, GCA/Precision Scientific Co., Chicago, IL) at 60°C for 48 h. The weight change of the larvae was calculated by taking the total weight of the larvae recovered / pot and dividing this number by the average number of larvae recorded for that trials control treatment (neonate or second

instar depending on treatment). Then the average initial starting weight of the larvae when they were infested was subtracted from this average weight to obtain the change in weight.

Beetle Recovery. Beetle emergence pots were covered with meshes to prevent beetle escapes 1-2 d after the 15-day larval recovery to monitor adult emergence. Pots were checked for adults every one to two days and until two weeks after the last beetle was found. Control maize plants were allowed to grow for the whole experiment. The mesh was secured around the base of the maize plants with plastic zip ties. The *S. faberi* control plants were cut down to 4-5 cm above soil level and covered when the first adult was found on the maize control that was infested with the same larval stage. All adults collected were stored in 95% ethanol and kept until sex, head capsule width and dry weight could be recorded as described above.

Statistical Analysis. The larval, weight change, and head capsule width data were analyzed as a randomized complete block split in space design by using the PROC MIXED procedure of the statistical package SAS (2002-2003 SAS Institute Inc. Cary, NC, USA).

3.2 Results

Glyphosate Sprayed *S. faberi*.

Neonate larval recovery. Treatment ($F = 24.05$; $df = 6, 48$; $P < 0.0001$), treatment \times sample date ($F = 2.24$; $df = 12, 112$; $P = 0.0141$) and treatment \times trial \times sample date ($F = 2.67$; $df = 12, 112$; $P = 0.0034$) significantly influenced the number of larvae recovered. The main effect of trial ($F = 2.54$; $df = 1, 4$; $P = 0.1863$) was not significant justifying the combination of both trials. A significant number of larvae were recovered from the

live *S. faberi* and maize controls (treatments 1 and 13) (Fig. 1). The first sample date did not significantly differ between the maize and *S. faberi* controls while the second and third sample dates did, with maize larval numbers increasing and *S. faberi* numbers decreasing. Minimal number of larvae were recovered from treatments 11, 7, and 3, with the later recovering the majority on first sample date (average of 1/ pot).

Neonate weight change. Treatment ($F = 83.20$; $df = 6, 48$; $P < 0.0001$), treatment \times trial ($F = 2.35$; $df = 6, 48$; $P = 0.0452$), sample date ($F = 60.93$; $df = 2, 112$; $P < 0.0001$), and treatment \times sample date ($F = 37.58$; $df = 12, 112$; $P < 0.0001$) significantly influenced the change in weight of the larvae recovered. The main effect of trial ($F = 0.45$; $df = 1, 4$; $P = 0.5410$) was not significant. A significant positive weight change was recorded for the live *S. faberi* and maize controls (treatments 1 and 13) on the second and third recovery dates (10 d and 15 d), with no significant difference between the two on the 5 d recovery (Fig. 1). Neonate larvae throughout all three experiments did not have a significantly positive weight change on the controls for the first sample date (5 d). All larvae recovered for treatment 3 on the 5 d recovery had a negative change in weight. These results indicate that *S. faberi* roots become nutritiously deficient soon after being sprayed.

Second instar recovery. Trial ($F = 9.57$; $df = 1, 4$; $P = 0.0365$), treatment ($F = 16.29$; $df = 6, 48$; $P < 0.0001$), sample date ($F = 35.79$; $df = 2, 112$; $P < 0.0001$) and trial \times sample date ($F = 11.78$; $df = 2, 112$; $P < 0.0001$) had a significant influence on the number of larvae recovered. Trials were combined because the significant p-value, was expected with the replications 2 and 3 in trial one being infested with five and seven larvae respectively. The live controls (treatments 2 and 14) had no significant difference between the numbers of larvae recovered on the first sample date (figure 1). The second

and third sample dates were significant because the larvae on the maize started to complete development and pupate out, thus the recovered maize numbers declined. Besides the live controls (treatment 2 and 14), a significant number of larvae were only recovered on the first sample date (5 d) for the remaining treatments (4, 6, 8, 10 and 12). *Second instar weight change.* Treatment ($F = 3.99$ df = 6, 48; $P = 0.0026$), treatment \times trial ($F = 2.33$; df = 6, 48; $P = 0.0469$), treatment \times sample date ($F = 2.47$; df = 12, 112; $P = 0.0068$) and treatment \times trial \times sample date ($F = 2.20$; df = 12, 112; $P = 0.0164$) significantly influenced the change in weight of the larvae recovered. Treatment 2 and 14 were the only two treatments with larvae that had a positive change in weight (figure 1). The maize control (treatment 14) was the only treatment with a significant weight gain and this was on the third sample date (15 d). There was no significant difference between the weight change of maize and *S. faberi* controls on the first and second recovery dates (5d and 10 d).

Beetle emergence. Adults were only recovered from the *S. faberi* and maize controls. The controls infested with neonates averaged 0.4 ± 0.40 and 4.9 ± 1.08 beetle per pot for the *S. faberi* and maize controls respectively. The controls infested with second instar larvae averaged 0.3 ± 0.15 and 3.2 ± 0.74 beetles per pot for the *S. faberi* and maize controls respectively. The difference in the average number of beetles that emerged between the two controls is significant in both the neonate and second instar infestation. The average weight (mg) of the adults recovered from the neonate infested controls was 1.11 ± 0.0004 and 2.31 ± 0.15 for the *S. faberi* and maize respectively. The average weight (mg) of the adults recovered from the second instars infested control was 0.98 ± 0.08 and 2.41 ± 0.14 for the *S. faberi* and maize respectively.

Severed Maize.

Neonate recovery. Treatment ($F = 27.14$; $df = 5, 40$; $P < 0.0001$), sample date ($F = 5.44$; $df = 2, 96$; $P = 0.0058$), and treatment \times sample date all had a significant interaction on the number of larvae recovered. The main effect of trial ($F = 0.09$; $df = 1, 4$; $P = 0.7760$) was not significant so the two trials were combined. There was no significant difference between treatments 1 and 3 three on the first sample date, but there was on the second recovery date indicating that maize roots start to become nutritiously deficient between 5 and 10 after it is cut (figure 2). A significant number of larvae were recovered 10 d after the plant was severed (treatments 3 and 9). Treatment 9 and 11 both recovered more larvae than treatments 5 and 7 respectively, on the first sample date showing that the size of the root mass of the plant has an effect on the number of larvae recovered. The comparison of treatment 5 and 9 on the first sample date (5 d) was significant ($P = 0.0182$).

Neonate weight change. Treatment ($F = 33.21$; $df = 5, 40$; $P < 0.0001$), treatment \times trial ($F = 3.71$; $df = 5, 40$; $P = 0.0075$), sample date ($F = 21.11$; $df = 2, 96$; $p < 0.0001$), treatment \times sample date ($F = 24.54$; $df = 10, 96$; $P = < 0.0001$) and treatment \times trial \times sample date ($F = 2.59$; $df = 10, 96$; $P = 0.0080$) all had a significant influence on the change in weight of the larvae recovered. Treatments 3, 9, and 11 had only slight positive changes in weight including the sample dates that had significant numbers of larvae recovered (figure 2). The significant difference between treatments 1 and 3 on the second recovery date (10 d) indicates that severed maize is nutritiously deficient around 5 days after it is severed. The maize control was not significantly different from any of the other treatments on the first sample date.

Second instar recovery. Treatment ($F = 11.51$; $df = 5, 40$; $P < 0.0001$), sample date ($F = 103.17$; $df = 2, 96$; $P < 0.0001$), trial \times sample date ($F = 5.87$, $df = 2, 96$; $P = 0.0039$), and treatment \times sample date ($F = 3.86$; $df = 10, 96$; $P = 0.0002$) significantly influenced the number of larvae recovered. Treatments 2 and 4 were the only two treatments to have a significant number of larvae recovered on the second sample date (10 d) (figure 2). The live maize control (treatment 2) had decreasing larval recovery numbers on the second and third sample date (10 and 15 d) because of larvae completing development and entering pupation. Treatments 2 and 4 had no significant difference between them on the first recovery date, but there was a significant difference between the live control (treatment 2) and the plants that were severed 5 days before they were infested (treatments 6 and 10). This indicates that maize starts to become unsuitable for larval survival between 5 and 10 after it is severed.

Second instar weight change. The main effect of treatment ($F = 11.79$, $df = 5, 40$; $P < 0.0001$) had a significant influence on the number of larvae recovered. Treatment 2 and treatment 4 were not significantly different on the first sample date and were the only two treatments to have a positive change in weight change on this sample date (figure 2). Based on these results and the negative weight change in treatments 6, 8, 10 and 12, severed maize will only support a positive change in weight in second instars for five days after the plants are severed.

Beetle emergence. For the treatments infested with neonates the maize control plants were the only ones that recovered adults (7.8 ± 1.5 average beetles / pot). Adults were recovered from all treatments that were infested with second instars but only treatments two and four recovered adults from both trials. The control (treatment 2) recovered an

average of 6.4 ± 0.6 beetles per pot, which was significantly greater than all the other treatments. Treatment 4 recovered 1.9 ± 0.7 beetles per plot, which was significantly greater than the remaining treatments. Treatments six, eight, ten and twelve had no significant difference between them averaging less than one beetle recovered per pot. The control (treatment 2) had the highest average beetle dry weight of all the treatments (2.21 ± 0.12 mg).

Glyphosate Sprayed Maize.

Neonate larval recovery. Treatment ($F = 25.95$; $df = 5, 40$; $P < 0.0001$), sample date ($F = 4.76$; $df = 2, 96$; $P = 0.0107$), and treatment \times sample date ($F = 2.76$; $df = 10, 96$; $P = 0.0050$) significantly affected the number of larvae recovered. Treatment 1, 3, 5 and 9 did not significantly differ in the number of larvae recovered on the first recovery date indicating that glyphosate sprayed maize will support larval survival for 10 days (figure 3). Treatment 9 had more larvae recovered than treatment 5 showing a slight but non-significant advantage to a larger root mass.

Neonate weight change. Treatment ($F = 17.30$; $df = 5, 40$; $P < 0.0001$), sample date ($F = 5.96$; $df = 2, 96$; $P = 0.0036$) and treatment \times sample date ($F = 7.50$; $df = 10, 96$; $P < 0.0001$) significantly affect the weight change in the larvae recovered. The live control (treatment 1) had a positive increase in weight change over all three-sample dates while treatment 3 had a slightly positive but decreasing weight change over the three sample dates. Treatments 3 and 11 proved that larvae can have a positive weight change for up to 15 day on glyphosate sprayed maize.

Second instar larval recovery. Treatment ($F = 32.00$; $df = 5, 40$; $P < 0.0001$), treatment \times sample date ($F = 3.07$; $df = 5, 40$; $P = 0.0194$), sample date ($F = 142.35$; $df = 2, 96$; $P < 0.0001$), treatment \times sample date ($F = 6.51$; $df = 10, 96$; $P < 0.0001$) and treatment \times trial \times sample date significantly affected the number of larvae recovered. The live control (treatment 2) was not significantly different from treatments 4 and 10 on the first sample date (figure 3). The control was significantly different from the treatments 8 and 12 indicating that the glyphosate sprayed maize plants begin to become unsuitable for WCR survival between 10 and 15 days after they are sprayed. The difference in root mass had a significant affect in the number of larvae recovered between treatments 6 and 10 on the first sample date (5 d). There was also no significant difference in the number of larvae recovered from treatments 2 and 4 on all three-sample dates. This is the case because some larvae have completed there development and begun to pupate on the maize control (treatment 2).

Second instar weight change. The main effect of treatment ($F = 5.64$; $df = 5, 40$; $P = 0.0005$) significantly affected the change in larval weight. There was no significant difference in weight change between treatments 2 and 4 on all three-sample dates. Treatments 6 and 10 had a slightly positive weight gain for the first sample dates. The result show that depending on which treatment glyphosate sprayed maize can support second instar positive weight gain for 10 to 15 d. Negative weight change was recorded though for all larvae that were infested on plants that were sprayed 10 days before infestation.

Beetle emergence. The maize controls were the only treatments on which adults were recovered. The neonate infested control averaged 4.0 ± 1.48 beetles per plot and the

second instar infested control averaged 4.0 ± 1.22 beetles per plot. The average beetle dry weight (mg) for the neonate and second instar infested controls were 2.09 ± 0.05 and 2.11 ± 0.15 respectively.

3.3 Discussion

In the current study, when *S. faberi* was sprayed with glyphosate, neonate western corn rootworm survival and weight gain was minimal (Fig. 1). Second instar western corn rootworm larvae had a significant number of larvae recovered on the first sample date (5 d), but there was no positive weight gain for any *S. faberi* treatments sprayed with glyphosate on any sample date (Fig. 1). These results indicate that within the first five days after *S. faberi* was sprayed with glyphosate, it became nutritionally unsuitable to both neonate and second instar western corn rootworm larvae. Recovery of second instars on the first sample date can be attributed to the ability of the larger larvae to withstand a longer period of starvation. The ability living *S. faberi* to support neonate and second instar larvae for a positive weight change on all three sample dates indicates its suitability as an alternate host.

Severed maize supported neonate and second instar larvae slightly better than that of *S. faberi*, which had been sprayed with glyphosate. Roots of severed plants supported larval survival for 5-10 d and a positive weight change for 5 days. Maize sprayed with glyphosate supported larval survival and weight gain best out of the three experiments. The number of larvae recovered 10 d after the maize was sprayed did not significantly differ from the living maize control for both neonate and second instar larvae (Fig. 3). Overall, Fig. 3 appears to indicate that maize roots became nutritionally unsuitable

between 10 and 15 d after the plants were sprayed with glyphosate in the current greenhouse experiment, which was quite different from the response of western corn rootworm larvae to the roots of *S. faberi* sprayed with glyphosate (Fig. 1). Glyphosate sprayed maize roots can support a positive change in weight for both neonate and second instar larvae for up to 15 d, depending on the treatment. The difference between the experiments in which maize was severed and the experiment where maize was sprayed with glyphosate might be explained by the time of actual plant death. Since the severed maize plant was severed near ground level and below the growing point, plant death was immediate, while the glyphosate-sprayed maize plants did not show signs of total plant death until 5 to 7 days after they were sprayed. The reason for the nutritional difference between the glyphosate-sprayed maize and glyphosate-sprayed *S. faberi* roots is unknown. Some possible factors may include the difference in the diameter of the roots, the suitability of the host plant in general, or the time needed for the glyphosate to kill the plant. Time to initial herbicide symptoms in *S. faberi* was only about 3 d.

Adult emergence in all studies was minimal and was found primarily in living control plants. Only one of the three experiments had adults emerge from any treatment other than living plants. The severed maize experiment had adults recovered in all second instar infested treatments in trial one, which was understandable when we examined the HCW of the larvae used to infest these treatments. In trial one, the first three replications were infested with larvae that were allowed to feed on maize roots from 9 to 11 d before they were used to infest the maize treatments. The HCW of these larvae indicated that they were actually third instars. Adults were only recovered from these three reps excluding the living plants. The fact that adults were recovered in Treatment 4

(severed on the day of infestation with second instar larvae) of trial two cannot be explained because HCW of these larvae revealed they were second instars. The only explanation is these roots are more nutritious than any other non-control treatments. The lack of any adults being recovered from the non-control treatments in the glyphosate sprayed treatments is surprising since it supported larval survival and positive weight change longer than the severed maize plants.

Fisher (1984), showed in a field experiment that when maize plants were severed at the base when insects were in the pupae stage of western corn rootworm development, the number of adults emerging between severed and living plants were not significantly different. Adult emergence of the severed plants was accelerated when the plants were severed at ground level. Our results from the severed maize experiment would tend to support these findings as long as the majority of the larvae are in the third instar stage or the pupa stage. The few cases where we evaluated early third instar larvae, adults were produced. However, when larvae were neonates or second instars, adults were generally not produced.

The amount of time the roots of maize and *S. faberi* plants remained nutritious may add some insight into the Moeser and Vidal (2004a, b) experiments. Moeser and Vidal (2004a, b) assumed that severed maize and alternate host roots in their experiments were nutritiously sufficient for up to six days. Our results indicate that this could have been the case for their maize varieties. Severed maize roots became nutritionally inadequate sometime between 5 and 10 d after they were severed. Our results for the *S. faberi* experiment indicated that within the first five days after the plants were sprayed with glyphosate they become nutritiously deficient. The results of Moser and Vidal

(2004b) may have been confounded by any differences between different alternate hosts in how fast that they senesce.

In conclusion, the results from this study will add insight into the interaction of maize, alternate hosts and western corn rootworm after a post-emergence herbicide application. These results also indicate that *S. faberi* roots become unsuitable for western corn rootworm development earlier than most rootworm scientists may have anticipated. Although this data was only with maize and *S. faberi*, this information may be applicable to other grassy weeds as well.

Table 10. Glyphosate sprayed *S. faberi* treatments

Treatments 1 ^a and 2	Live <i>S. faberi</i> control
Treatments 3 and 4	<i>S. faberi</i> sprayed on infestation day
Treatments 5 and 6	<i>S. faberi</i> sprayed 5 d before infestation day
Treatments 7 and 8	<i>S. faberi</i> sprayed 10 d before infestation day
Treatments 9 and 10	<i>S. faberi</i> planted 5 d early and sprayed 5 d before infestation day
Treatments 11 and 12	<i>S. faberi</i> planted 10 d early and sprayed 10 d before infestation day
Treatments 13 and 14	Live maize control

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^aOdd numbered treatments were infested with 30 neonate larvae and the even numbered treatments were infested with 8 second instar larvae (10 for maize). Treatments descriptions are similar for glyphosate sprayed maize or severed maize with the exception of Treatments 13 and 14 being omitted.

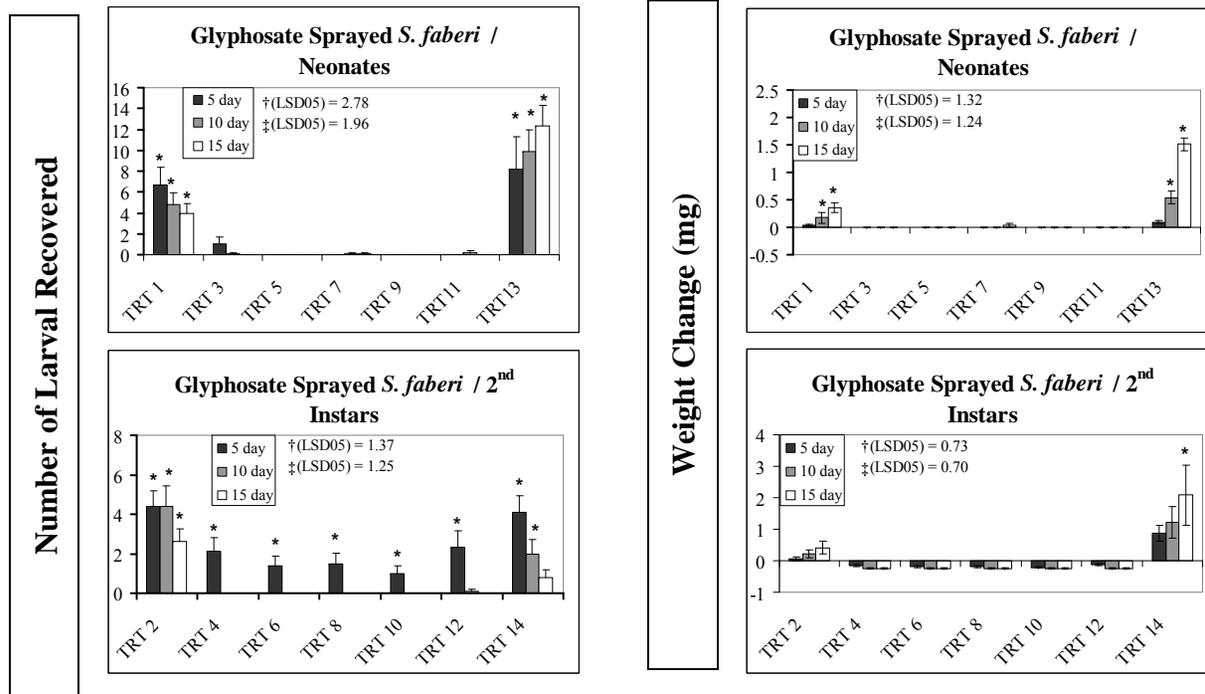


Fig. 1. Graphs of glyphosate sprayed *S. faberi* for larval recovery and weight change. * indicates a significant number of larvae recovered or a significant weight change. † indicates the least significant difference ($P = 0.05$) when comparing bars with the same recovery date but different treatments. ‡ indicates the least significant difference ($P = 0.05$) when comparing bars within the same treatments but different recovery dates.

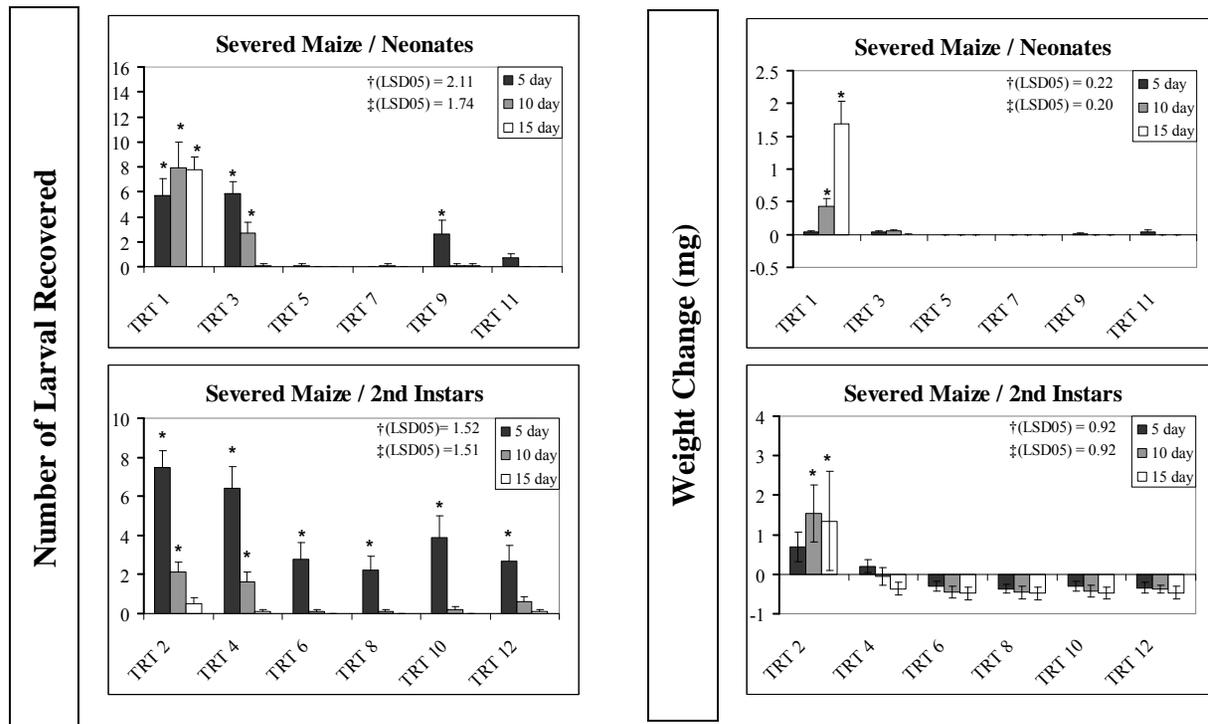


Fig. 2. Graphs of severed maize for larval recovery and weight change. * indicates a significant number of larvae recovered or a significant weight change. † indicates the least significant difference ($P = 0.05$) when comparing bars with the same recovery date but different treatments. ‡ indicates the least significant difference ($P = 0.05$) when comparing bars within the same treatments but different recovery dates.

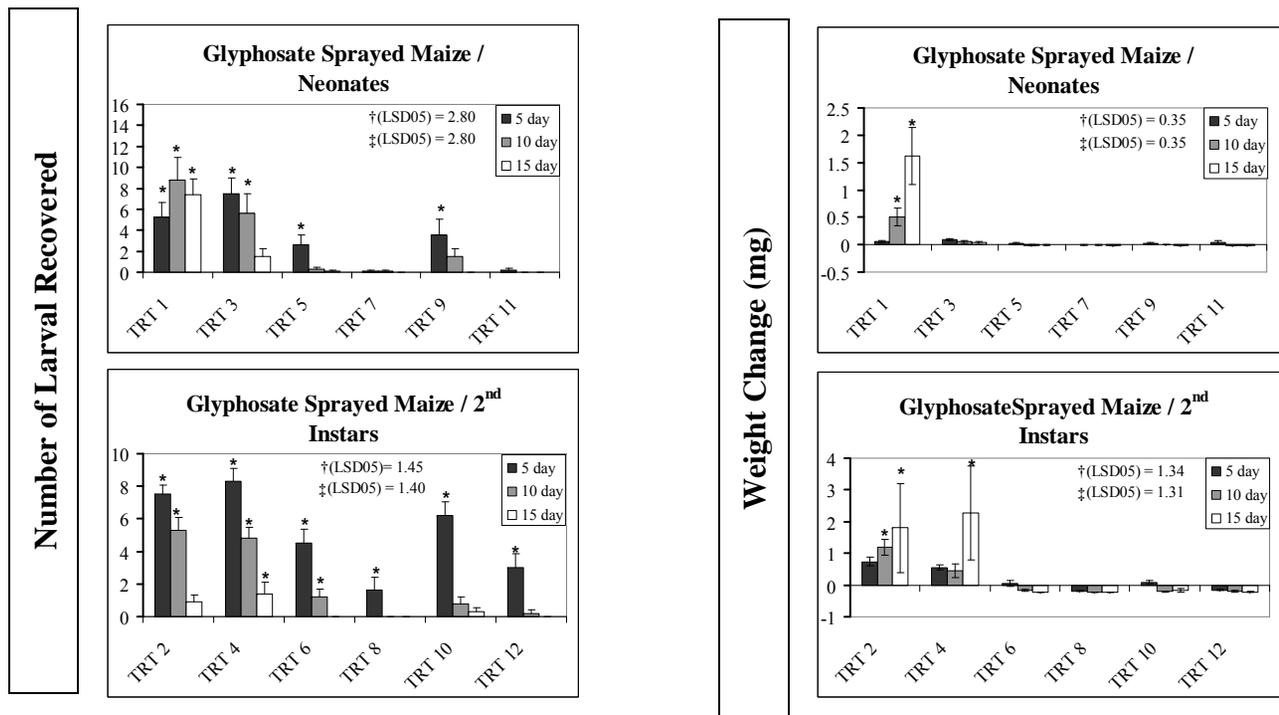


Fig. 3. Graphs of glyphosate sprayed maize for larval recovery and weight change. * indicates a significant number of larvae recovered or a significant weight change. † indicates the least significant difference ($P = 0.05$) when comparing bars with the same recovery date but different treatments. ‡ indicates the least significant difference ($P = 0.05$) when comparing bars within the same treatments but different recovery date

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