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ORIGINAL CONTRIBUTION

Larval mortality and development for rotation-resistant and rotation-susceptible populations of western corn rootworm on Bt corn

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Abstract

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is one of the most economically important insect pests threatening the production of corn, *Zea mays* (L.), in the United States. Throughout its history, this insect has displayed considerable adaptability by overcoming a variety of pest management tactics, including the cultural practice of annual crop rotation. Since first reported in Illinois in the late 1980s, populations of the rotation-resistant western corn rootworm have spread over a wide area of the eastern Corn Belt. Currently, little information is available concerning the interaction of rotation resistance with the use of genetically modified corn expressing insecticidal toxins from *Bacillus thuringiensis* Berliner (Bt), a popular tactic for preventing larval injury and its associated yield loss. The goal of this greenhouse experiment was to determine whether rotation-resistant and rotation-susceptible western corn rootworm larvae differ with respect to survival or development when exposed to single- or dual-toxin (pyramided) Bt corn. Individual corn plants were infested with 225 near-hatch eggs at the V5 (five leaf collar) growth stage. Larvae developed undisturbed on the root systems for 17 days, after which they were recovered using Berlese–Tullgren funnels. Surviving larvae were counted to estimate mortality, and head capsule widths were measured to assess development. Rotation-resistant and rotation-susceptible larvae had statistically similar mean levels of mortality and head capsule widths when exposed to both single-toxin (Cry3Bb1 or Cry34/35Ab1) and pyramided (Cry3Bb1+ Cry34/35Ab1) Bt corn, suggesting that these two populations do not differ with respect to survival or development when exposed to Bt corn. Additionally, the statistically similar mean levels of mortality for larvae exposed to single-toxin and pyramided Bt corn suggest that pyramided Bt hybrids containing the Cry3Bb1 and Cry34/35Ab1 toxins do not result in additive mortality for western corn rootworm larvae. Implications for management of this economically important pest are discussed.

Introduction

Corn, *Zea mays* (L.), is an important grain crop cultivated globally for a variety of uses. The United States

accounted for approximately 37% of the 9.6×10^{11} kg of corn produced globally in 2013 (USDA FAS [United States Department of Agriculture Foreign Agricultural Service] 2014). One of the most

economically important insect pests threatening corn production in the United States is the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (Coleoptera: Chrysomelidae). Yield losses and control costs associated with corn rootworm species – which also include the northern (*Diabrotica barberi* Smith & Lawrence) and southern (*Diabrotica undecimpunctata howardi* Barber) corn rootworms – exceed \$1 billion annually in the United States (Metcalf 1986). Western corn rootworm larvae feed on the root tissue of corn plants, which can result in impaired photosynthesis (Godfrey et al. 1993; Riedell and Reese 1999), reduced uptake of water (Godfrey et al. 1993) and nitrogen (Spike and Tollefson 1989); colonization by phytopathogenic or saprophytic fungi (Bryson et al. 1953; Kurtz et al. 2010) and plant lodging (Spike and Tollefson 1991). Although yield loss due to root injury is highly variable (Gray and Steffey 1998), Tinsley et al. (2013) estimated that for every node (i.e. circle of roots) consumed by corn rootworm larvae, a yield loss of approximately 15% can be expected.

Throughout its pest history, the western corn rootworm has demonstrated a high degree of adaptability, as it has overcome a variety of pest management strategies (Gray et al. 2009). This insect has evolved resistance or reduced susceptibility to carbamate (Meinke et al. 1998), organophosphate (Ball 1968; Call et al. 1977; Meinke et al. 1998) and organochlorine (Ball and Weekman 1962; Hamilton 1965) insecticides. Annual crop rotation (rotating corn with a non-host crop) was highly effective for managing the western corn rootworm since it was first recommended in the early 1900s (Gillette 1912). However, much of the east-central Corn Belt now hosts a variant western corn rootworm with resistance to crop rotation (Levine et al. 2002). Additionally, field-evolved resistance to genetically modified corn expressing an insecticidal toxin (Cry3Bb1) from *Bacillus thuringiensis* Berliner (Bt) has been confirmed for this insect (Gassmann et al. 2011). Unfortunately, little information is available concerning the potential interaction of rotation-resistant populations of the western corn rootworm with alternate management tactics. One example of this type of interaction is the relative effectiveness of Bt toxins against rotation-resistant and rotation-susceptible populations of the western corn rootworm. Understanding this type of interaction may allow applied entomologists to refine management recommendations.

Gray et al. (2007) evaluated a number of Bt hybrids targeting the western corn rootworm – all hybrids expressed the same Bt toxin (Cry3Bb1), which was inserted using the same transgenic event

(MON-ØØ863-5, Monsanto Co., St. Louis, MO). During 1 year of their experiment (2006), two trial sites were evaluated (Monmouth and Urbana, IL). Urbana is located in a region of Illinois that has historically had high populations of the rotation-resistant western corn rootworm. However, at Monmouth, the likelihood of a large rotation-resistant population of this pest was much lower in 2006. Although root injury for the non-Bt untreated check was similar at both locations, root injury for the Bt hybrids was approximately 105% greater at the Urbana site. The authors hypothesized that rotation-resistant and rotation-susceptible larvae may differ in their capacity to injure Bt roots. However, the experimental design used by Gray et al. (2007) was not constructed in a manner that could test this hypothesis explicitly – Bt hybrids at Monmouth and Urbana may have been subjected to feeding by both rotation-resistant and rotation-susceptible larvae in unknown proportions. The goal of the greenhouse experiment described here was to determine whether rotation-resistant and rotation-susceptible western corn rootworm larvae differ with respect to survival or development when exposed to corn expressing Bt toxins. A single-plant bioassay was used with techniques adapted from Gassmann et al. (2011).

Materials and Methods

Experimental design

This experiment was conducted during 2012 and 2013 using a two-way factorial arrangement in a completely randomized design. The first factor was western corn rootworm population and included two levels: (i) rotation resistant and (ii) rotation susceptible. This first factor is hereafter referred to as ‘population’. Larvae from each population were evaluated for survival and development on five corn hybrids, including a hybrid expressing Cry34/35Ab1 (Event DAS-59122-7, Pioneer 34P94, DuPont Pioneer, Johnston, IA), its near-isoline (Pioneer 34P89, DuPont Pioneer), a hybrid expressing Cry3Bb1 (Event MON-88Ø17-3, DKC61-19, Monsanto Co., St. Louis, MO), its near-isoline (DKC61-22, Monsanto Co.), and a hybrid expressing both Cry3Bb1 and Cry34/35Ab1 (Events MON-88Ø17-3 and DAS-59122-7, DKC61-21, Monsanto Co.) – DKC61-19 and DKC61-21 shared a common near-isoline (DKC61-22). This second factor is hereafter referred to as ‘treatment’. In total, three runs of this experiment were performed. For each run, four replications of each population × treatment combination were used.

Seed preparation and planting information

All seed used during this experiment was treated with a neonicotinoid insecticidal seed treatment (either clothianidin or thiamethoxam) at 0.25 mg active ingredient/seed. These insecticidal treatments were removed because the primary focus of this experiment was to examine mortality caused by the Bt toxins being evaluated. Seeds were first placed in a 1% detergent solution (Dawn Ultra Concentrated Dish Soap, Proctor & Gamble, Cincinnati, OH) and agitated gently for 20 min. Following agitation, the seeds were rinsed thoroughly under running water. This wash/rinse procedure was repeated for a total of three cycles. Washed seeds were allowed to dry for 12 h, after which they were soaked in a 10% bleach solution (Clorox Regular Bleach, The Clorox Co., Oakland, CA). While soaking in the bleach solution, the seeds were stirred periodically. After soaking for approximately 1 h, the seeds were rinsed thoroughly under running water and allowed to dry for at least 24 h prior to planting.

This experiment was conducted at the Natural Resources Studies Annex greenhouse on the University of Illinois campus in Champaign, IL. The greenhouse was maintained at 25°C with 16 h of light each day. For each run, 40 single-plant pots were prepared (2 populations × 5 hybrids × 4 replications/run). Pots were made of white polypropylene and had a volume of 950 ml (Item No. 81144, US Plastic Corp., Lima, OH). Four 3-mm holes were drilled into the bottom of each pot for drainage. The pots were then filled with 750 ml of a 1 : 1 mixture of potting soil (Sunshine Mix LC1, Sun Gro Horticulture Canada Ltd., Vancouver, BC) and sterilized topsoil. Prior to planting the seeds, each pot was fertilized with 4 g of a 14-14-14 (N-P-K) slow-release fertilizer (Osmocote Smart-Release Plant Food, The Scotts Miracle-Gro Co., Marysville, OH). A single seed was planted in the centre of each pot at a depth of 1 cm. Pots were watered as needed to maintain a uniform level of moisture, with a maximum of 50 ml of water per day. Periodically, all of the plants were trimmed so that their overall height did not exceed 30 cm – trimming the plants reduced their overall water requirement and helped maintain consistent moisture.

Egg preparation and infestation technique

The western corn rootworm eggs used during this experiment were provided by the USDA ARS North Central Agricultural Research Laboratory (NCARL) at Brookings, SD. The rotation-resistant and

rotation-susceptible populations were identified as 'Spencer' (initially collected from Illinois, in culture since 2010) and 'Whitlock' (initially collected from South Dakota, in culture since 1996), respectively (C. N. Nielson, USDA ARS NCARL, personal communication). Upon receipt, the eggs and their soil matrix were soaked in water for approximately 30 min. To separate eggs from the soil, the solution was poured through a stainless steel mesh sieve with 250-µm pores. The eggs were rinsed under running water for 5 min to remove all soil. The eggs were then placed into a 950-ml container filled with 750 ml of water. We used water as a medium for post-diapause egg development because previous attempts using moist soil resulted in excessive fungal growth and reduced egg viability. The eggs were examined regularly to monitor their development. To prevent fungal growth in the egg containers, approximately 75% of the water was replaced daily. Eggs were ready to hatch (i.e. neonate larvae were visible within the eggs) after approximately 21 days of storage at room temperature.

Infestation of greenhouse plants by near-hatch eggs was timed to coincide with the V5 (five leaf collar) growth stage. For each plant, 225 eggs (measured volumetrically) were transferred from the egg containers into a 1.5-ml microcentrifuge tube. To infest each plant, a 10-cm-long hole was bored into the soil approximately 2.5 cm from the base of the plant. The pre-measured volume of eggs was transferred from the microcentrifuge tube to the hole using a plastic pipette. After depositing the eggs, the infestation site was closed by gently pinching together the surface of the soil near the top of the hole. Larvae were allowed to develop undisturbed on each plant for 17 d – Gassmann et al. (2011) noted that this duration was sufficient to allow the fastest developing larvae to reach the final developmental stage (instar) prior to pupation.

Larval extraction and processing

To extract larvae from each pot, plants were cut near the soil surface and the pots were transported to the Agricultural Engineering Farm near Urbana, IL. The contents of each pot (soil and root tissue) were gently removed and placed onto a square piece of aluminium screen (20 cm on each side) with 45 holes/cm². This screen was then placed into the upper chamber of a collapsible Berlese–Tullgren funnel trap (Item No. 2832; BioQuip Products Inc., Rancho Dominguez, CA). These traps are commonly used to extract insects and other arthropods from leaf litter or soil and

operate by utilizing a heat source placed above the sample to stimulate mobile organisms to move away from the heat and towards a collection container at the bottom of the trap. For this experiment, the collection container was a Whirl-Pak bag (Item No. 1114B, BioQuip Products Inc.) filled with 50 ml of 85% ethanol, and the heat source was a 40 W incandescent light bulb. Larvae were extracted continuously for a period of 72 h, after which the number of larvae present in each sample was recorded.

From each sample, a subsample of three larvae was selected at random for head capsule measurement. Western corn rootworm larvae develop through three instars – these developmental stages can be differentiated by measuring the width of their head capsule. First, second and third instar larvae have head capsule widths measuring within the ranges of 0–270, 270–410 and 410–580 μm , respectively (Hammack et al. 2003). Head capsule widths were determined for each larva by measuring the widest portion of the head. A dissecting microscope (Model MZ125, Leica Microsystems Inc., Buffalo Grove, IL) with an ocular reticle was used – a magnification of 10X was selected to achieve an accuracy of 10 μm .

Statistical analysis

To standardize comparisons between western corn rootworm populations, the relative corrected mortality for each Bt sample was determined using formulae adapted from Abbott (1925). This metric is ‘relative’ because it is calculated for each population independently and ‘corrected’ because it accounts for natural mortality not caused by exposure to Bt toxins. First, the proportional survival for each sample was calculated using the formula:

$$s_{ti} = \frac{l_{ti}}{r} \quad (1)$$

Where s_{ti} was the proportional survival for sample i of treatment t , l_{ti} was the number of larvae recovered from sample i of treatment t , and r was the infestation rate. Next, the mean proportional survival for each near-isoline treatment for each population was calculated using the formula:

$$\bar{s}_t = \left(\sum_{i=1}^{n_t} s_{ti} \right) \div n_t \quad (2)$$

where $\bar{s}_t$ was the mean proportional survival for near-isoline treatment t , s_{ti} was the proportional

survival for sample i of near-isoline treatment t , and n_t was the total number of samples for near-isoline treatment t . Finally, the corrected mortality for each sample of the Bt treatments for each population was calculated using the formula:

$$m_{ti} = \frac{\bar{s}_t - s_{ti}}{\bar{s}_t} \quad (3)$$

where m_{ti} was the corrected mortality for sample i of Bt treatment t , $\bar{s}_t$ was the mean proportional survival for near-isoline treatment t , and s_{ti} was the proportional survival for sample i of Bt treatment t .

Response variables (proportional survival for near-isoline treatments, relative corrected mortality for Bt treatments and head capsule widths) were analysed using SAS 9.2 (SAS Institute Inc., Cary, NC). To stabilize variances and meet the assumptions of analysis of variance, response variables were analysed using a square-root transformation. Statistical tests for fixed effects were performed using PROC MIXED. Fixed effects included population, treatment, and their interaction and were declared significant at $P \leq 0.05$. Means were compared using the LSMEANS option of PROC MIXED and were declared significantly different at $P \leq 0.05$.

Results

Because a significant interaction effect was never documented for any response variable, means are only presented for main effects. Mean proportional survival was only analysed for near-isoline treatments. A significant effect of population on mean proportional survival was observed (table 1), with rotation-resistant larvae averaging a 14.0% greater level of proportional survival than rotation-susceptible larvae (table 2). No significant effect of treatment on mean proportional survival was observed (table 1), indicating that mean proportional survival was similar for both near-isoline treatments (table 2).

No significant effect of population, treatment, or their interaction was observed for mean relative corrected mortality for Bt treatments (table 1). With respect to the effect of population, mean relative corrected mortality levels for both rotation-resistant and rotation-susceptible larvae differed by less than 3.0% and were statistically similar (table 3). For the effect of treatment, mean relative corrected mortality levels ranged from 68.8 to 71.8% and were statistically similar across all Bt treatments, including the Cry3Bb1 + Cry34/35Ab1 dual-toxin treatment (table 3).

Table 1 Statistical tests¹ of fixed effects for response variables analysed

Response variable	Effect ²	df _N	df _D	F	P
Proportional survival for near-isoline treatments	Population	1	2	39.2	0.02
	Treatment	1	2	1.3	0.37
	Population × treatment	1	2	4.2	0.18
Relative corrected mortality for Bt treatments	Population	1	2	1.4	0.36
	Treatment	2	4	0.3	0.74
	Population × treatment	2	4	0.5	0.62
Head capsule widths	Population	1	2	2.0	0.30
	Treatment	4	8	12.1	<0.01
	Population × treatment	4	8	3.0	0.09

¹Statistical tests were performed using PROC MIXED of SAS 9.2. Data were analysed using a square-root transformation.

²Effects were declared significant at $P \leq 0.05$.

Table 2 Mean¹ proportional survival for near-isoline treatments for the effects of population and treatment

Effect	Level	Proportional survival (%) ²
Population	Rotation resistant	52.7 a
	Rotation susceptible	38.7 b
Treatment	Cry3Bb1 near-isoline	46.5 a
	Cry34/35Ab1 near-isoline	44.8 a

¹Mean comparisons were made using PROC MIXED of SAS 9.2. Means within a column for an individual effect that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analysed using a square-root transformation; actual means are reported.

²Proportional survival represents the number of larvae recovered divided by the infestation rate (225 eggs/plant).

Table 3 Mean¹ relative corrected mortality for Bt treatments for the effects of population and treatment

Effect	Level	Relative corrected mortality (%) ²
Population	Rotation resistant	68.5 a
	Rotation susceptible	71.4 a
Treatment	Cry3Bb1	68.8 a
	Cry34/35Ab1	69.3 a
	Cry3Bb1+ Cry34/35Ab1	71.8 a

¹Mean comparisons were made using PROC MIXED of SAS 9.2. Means within a column for an individual effect that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analysed using a square-root transformation; actual means are reported.

²Relative corrected mortality represents the mortality caused by the Bt treatments corrected for natural mortality relative to each population.

Only the effect of treatment on mean head capsule width was statistically significant – the effect of population and the interaction of population and treatment were not (table 1). With respect to the effect of population, mean head capsule widths for rotation-resistant and rotation-susceptible larvae differed by only 5 µm and were statistically similar (table 4). For the effect of treatment, mean head capsule widths for larvae on near-isoline treatments were significantly greater than mean head capsule widths for larvae on Bt treatments (table 4). Additionally, no significant differences were observed in mean head capsule widths between the near-isoline treatments or among the various Bt treatments (table 4).

Discussion

Proportional survival of larvae for near-isoline treatments was analysed to determine whether the level of natural mortality differed between the two populations of western corn rootworm evaluated during this experiment. Determining proportional survival for each population was necessary to standardize comparisons of mortality caused by treatments containing Bt toxins. Because proportional survival between rotation-resistant and rotation-susceptible larvae differed significantly (table 2), the decision to use a relative parameter to analyse corrected mortality caused by the Bt toxins was justified. It is unclear why these two populations differed with respect to proportional survival on near-isoline treatments. The rotation-resistant population was represented by the Spencer colony, which has been in culture since 2010 (ca. 2 generations). In contrast, the Whitlock colony was

Table 4 Mean¹ head capsule widths for the effects of population and treatment

Effect	Level	Head capsule width (µm)
Population	Rotation resistant	245 a
	Rotation susceptible	240 a
Treatment	Cry3Bb1	227 b
	Cry3Bb1 near-isoline	264 a
	Cry34/35Ab1	235 b
	Cry34/35Ab1 near-isoline	265 a
	Cry3Bb1+ Cry34/35Ab1 ²	222 b

¹Mean comparisons were made using PROC MIXED of SAS 9.2. Means within a column for an individual effect that share a similar letter do not differ significantly ($P \leq 0.05$). Data were analysed using a square-root transformation; actual means are reported.

²The near-isoline for this dual-toxin treatment was the Cry3Bb1 near-isoline.

used to represent the rotation-susceptible population – this colony has been in culture since 1996 (ca. 16 generations). Although the duration of time spent in culture between these two colonies varies substantially, it is unlikely that this difference contributed to the observed difference in proportional survival. Kim et al. (2007) used microsatellite loci to compare genetic diversity among various colonies maintained at USDA ARS NCARL (including some wild-type populations as well). Their findings demonstrated that western corn rootworm colonies maintained in culture for up to 22 generations could not be differentiated from wild-type populations based on genetic diversity.

One of the principal benefits associated with Bt corn is its demonstrated ability to cause substantial mortality among western corn rootworm larvae (Storer et al. 2006; Hibbard et al. 2010). During this experiment, relative corrected mortality was analysed to determine whether rotation-resistant and rotation-susceptible larvae differed with respect to survival on Bt corn. While the levels of mortality due to Bt toxin exposure observed in this experiment may appear lower than expected (68.8–71.8 for the various Bt treatments, table 3), they are not out of the range of what has been reported previously (as summarized by Gassmann 2012). Of primary interest is how our results compare with those of Gassmann et al. (2011), as our techniques were modelled after their methods. For the offspring of beetles collected from their 'control' fields (i.e. areas without western corn rootworm populations suspected to be resistant to Cry3Bb1), Gassmann et al. (2011) reported levels of mortality ranging from 68 to 94% or 75 to 100% when exposed to Cry3Bb1- or Cry34/35Ab1-expressing corn, respectively. Because the levels of mortality we observed are very near the ranges reported by Gassmann et al. (2011), we feel that our data are of sufficient quality to draw meaningful conclusions. The lack of a significant effect of population on relative corrected mortality (table 3) suggests that mortality caused by the Bt toxins evaluated during this experiment (Cry3Bb1 and Cry34/35Ab1) does not differ between rotation-resistant and rotation-susceptible larvae. Additionally, no significant effect of treatment on relative corrected mortality was observed – larvae experienced similar levels of mortality when exposed to either single- or dual-toxin (i.e. pyramided) Bt corn (table 3). This observation suggests that larval mortality caused by each of the toxins expressed in the pyramided treatment was non-additive.

The demonstration of non-additive larval mortality associated with the pyramided treatment may have

implications for integrated resistance management. Onstad and Meinke (2010) suggested that the evolution of resistance to Bt corn by the western corn rootworm would be delayed if a pyramided, multiple-toxin strategy was adopted over a single-toxin approach. However, Onstad et al. (2011) questioned the value of a pyramided strategy for integrated resistance management if multiple-toxin Bt corn failed to increase mortality in the target pest when compared with single-toxin Bt corn. Recent estimates suggest that mortality caused by one such pyramided product – marketed as Genuity SmartStax (Monsanto Co.) or SmartStax (Dow AgroSciences LLC, Indianapolis, IN) – is not substantially greater than either of its constituent single toxins (Cry34/35Ab1 and Cry3Bb1) (USEPA OPPTS [United States Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances] 2011). Results from a field experiment evaluating adult emergence summarized by Head et al. (2014) support this conclusion as well. When averaged across sites, survivorship was 3.3872% and 1.6573% for the single-toxin treatments DAS-59122-7 (Cry34/35Ab1) and MON-88017-3 (Cry3Bb1), respectively (values derived from table 6 of Head et al. 2014). Survivorship for the dual-toxin treatment DAS-59122-7 × MON-88017-3 (Cry34/35Ab1+ Cry3Bb1) was 1.0699% when averaged across sites, which does not appear to be substantially lower than survivorship for the single-toxin treatment MON-88017-3.

Many experiments have documented delayed emergence of western corn rootworm adults when exposed to Bt corn (Storer et al. 2006; Murphy et al. 2010; Spencer et al. 2012), which has generally been attributed to slower than typical larval development. Larval head capsule widths were evaluated for a subsample of all larvae recovered during this experiment to determine whether rotation-resistant and rotation-susceptible larvae differed with respect to development on Bt corn. Mean head capsule widths were greatest for larvae on near-isoline treatments (table 4). This observation agrees with results presented by Hibbard et al. (2010), which demonstrated that larvae exposed to non-Bt corn typically had greater head capsule widths than those exposed to Bt corn. Mean head capsule widths for larvae from both populations were statistically similar (table 4), indicating that larvae from these two populations develop similarly when exposed to the treatments evaluated during this experiment.

Based on the results of their field experiment, Gray et al. (2007) hypothesized that rotation-resistant western corn rootworm larvae may cause more injury

to Bt corn than rotation-susceptible larvae. During this experiment, mortality and development were evaluated for larvae from rotation-resistant and rotation-susceptible populations. Because mean relative corrected mortality levels and head capsule widths were similar for both rotation-resistant and rotation-susceptible larvae, as well as a documented lack of any significant interaction between the effects of population and treatment for the response variables analysed, we conclude that any hypothesized difference in the ability of these two populations to injure roots expressing one or more Bt toxins is unrelated to survival or development.

The fundamental difference between rotation-resistant and rotation-susceptible populations of the western corn rootworm is the loss of ovipositional fidelity to cornfields among rotation-resistant females (Rondon and Gray 2004), which Pierce and Gray (2006) attributed to long term, strict annual rotation of corn and soya bean. To date, the use of genetic techniques to differentiate between rotation-resistant and rotation-susceptible beetles has been unsuccessful (Miller et al. 2006). However, Garabagi et al. (2008) noted that expression of cyclic GMP-dependent protein kinases is 25% greater in rotation-resistant females when compared with rotation-susceptible females. Genes for these proteins have been implicated in the regulation of behaviour in many animals. Recently, Curzi et al. (2012) reported that digestive enzymes play a role in facilitating rotation resistance. The authors noted that rotation-resistant females had increased constitutive expression of cathepsin L-like proteases, allowing them to overcome soya bean defences (cysteine protease inhibitors) and forage for a longer duration in soya bean than rotation-susceptible females. Apparently, adaptive changes in rotation-resistant adult behaviour or physiology have no correlation with the capacity of larvae to injure corn roots.

However, this greenhouse experiment focused on western corn rootworm larval survival and development, not potential to injure corn roots. Although no significant differences in survival or development on Bt corn between rotation-resistant and rotation-susceptible larvae were documented, other factors should be explored prior to concluding that no difference in capacity to injure corn roots exists between these two populations. One such example may be behavioural differences in root feeding. Clark et al. (2006) noted that western corn rootworm larvae exposed to Bt corn display two types of feeding behaviour – sedentary or roaming. Their findings suggest that larval feeding behaviour associated with exposure to Bt corn is

complex. Further research may be needed to identify whether other factors may contribute to a potential difference in the capacity to injure corn roots between rotation-resistant and rotation-susceptible larvae.

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