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The effect of stem diameter on European corn borer behavior and survival: potential consequences for IRM in Bt-corn

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Abstract

The ability of non-crop plants to support complete development of insect pests is an important factor for determining the impact of those plants on resistance management programs for transgenic crops. We assessed the effect of one physical factor, plant stem diameter, on the ability of plants to support full development of the European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), the target pest of transgenic Bt-corn. In the field, European corn borer larvae were significantly more likely to tunnel and survive in plants with larger stem diameters. Larvae were 40× more likely to survive on corn, the largest plant tested, compared to many of the smaller plants. In the laboratory, larvae were more likely to survive in and less likely to abandon the largest diet-filled artificial stems that varied only in stem diameter. In conditions simulating those that an ECB larvae would encounter upon abandoning a host, larvae survived up to three weeks and were able to locate corn as a new host with a significantly higher frequency than would be expected if they were foraging randomly. These results indicate that the probability of ECB larval survival to maturity on a plant other than corn is relatively low and thus these smaller stemmed non-corn plants may not make a substantial contribution to the pool of susceptible adults. Conversely, since more mature larvae are not as susceptible as neonates, any larvae that partially develop on non-corn plants and subsequently colonize Bt-corn may not be exposed to a lethal dose of the toxin. Since some proportion of the individuals that survive could be partially resistant heterozygotes the presence of non-corn host plants could facilitate the development of resistant ECB populations.

Introduction

The success of integrated resistance management (IRM) programs for transgenic crops hinges on the presence of susceptible adult insects to mate with any resistant individuals and a high enough toxin level to kill any partially resistant individuals that arise (Ostlie et al., 1997). Plants other than the protected crop that support full development of the pest can serve as an important source of those susceptible adults. Many pest insects oviposit eggs on host plants or host plant parts that will not allow them to complete development (Thompson, 1988). Plants that do not provide sufficient resource quantity or quality may still provide important temporal refuges as 'nursery plants' for the pest species by allowing partial development until more suitable host plants are available. These nursery plants can be a source of late instar larvae that subsequently colonize the protected crop. Since more mature larvae are generally less susceptible than neonates (Huang et al., 1999) any larvae that establish and partially develop on nursery plants may not be exposed to a lethal dose of the toxin if they subsequently colonize the target crop. This is a potentially serious problem because some proportion of the larvae that survive will be partially resistant heterozygotes (having one resistant allele) and the presence of these heterozygotes in the mating pool leads to the rapid development of resistance (Tabashnik, 1994). Thus, non-crop host plants that support complete pest de-

velopment play a positive role in IRM programs by providing a source of susceptible adults while nursery plants potentially play a negative role by harboring partially resistant heterozygote larvae. The physical characteristics of each plant can be one factor in determining the ability of the plant to support complete development of the pest and thus the plant's role in an IRM program.

This study focuses on *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), a major pest of corn known as the European corn borer (ECB). ECB has been reported to feed on 131 genera of 40 plant families (Caffrey & Worthley, 1927; Hodgson, 1928). In spite of its wide host range, ECB moths show marked oviposition preferences among host plant species, between plants of the same host species, and for different plant parts (Mason et al., 1996; Lupoli et al., 1990; Legg et al., 1986; Andrew & Carlson, 1976). ECB larvae also seem to express host plant preferences. Larvae are known to colonize alternative hosts after migrating away from plants that have become unsuitable for feeding or overwintering (Caffrey & Worthley, 1927; Crawford & Spencer, 1922; Hodgson, 1928; Hudon et al., 1989; Neiswander & Huber, 1927). Larval migration from corn was shown to be a major factor in population reduction (LeRoux et al., 1963). Differences in larval establishment and development among host species have been recorded (Legg et al., 1986; Hodgson, 1928; Huber et al., 1928; Caffrey & Worthley, 1927; Neiswander & Huber, 1927). Various factors have been suggested to account for these differences, including the inability of young larvae to feed on tough plant parts and stem diameters too small for tunneling (Beck, 1987; Neiswander & Huber, 1927).

Based on these previous studies it appears that ECB larval behavior is influenced by plant suitability. Host quality assessments are based on both physical and chemical host plant cues (Lupoli et al., 1990; Beck, 1987). Chemical and physical cues are almost inevitably correlated and thus it can be difficult to determine the relative importance of either class of cues. Here we studied the effect of one phyiscal factor, plant stem diameter, on ECB larval survival and behavior in the absence of other plant cues. Specifically we investigated the effect of stem diameter on the probability that ECB larvae will colonize, abandon, and survive on a host. Plant stem diameter is an attractive potential index of suitability because it is known or easily measured for all plants ECB is likely to encounter. We measured larval survival and behavior relative to plant diameter both in the field and in the laboratory and we assessed the behavior and survivorship of larvae placed in artificial 'stems' to isolate the effect of stem diameter from other factors. Based on the high proportion of larvae that escape from or abandon plants we also measured how long larvae can survive without the food and shelter of a plant and how likely ECB larvae foraging on the soil are to colonize corn compared with other host plants. Combining these behavior and survivorship studies we will elucidate the potential utilization of nursery plants by ECB and the probability of ECB larvae successfully colonizing corn following escape from a nursery host.

Materials and methods

To determine the effect of stem diameter on ability of a host plant to support complete development, the survival of ECB larvae was assessed both in the field on plants and in the laboratory on artificial stems. The effect of stem diameter on the probability of larval escape was also measured using the artificial stems. Because escape rates were relatively high from some stem diameters, ECB larvae were assessed for their longevity when held without the food or shelter that plants provide and for their ability to locate a corn plant while foraging on the soil.

All ECB larvae and adults with the exception of the low level of natural infestation on the plants in the field came from a colony in the Department of Plant Breeding at Cornell University (Chareinsuk, 1983). All field studies were carried out at the Homer C. Thompson research farm near Freeville, New York.

Larval survival in plants. ECB survival following infestation was measured and analyzed as a function of stalk diameter for corn, three non-corn crops (oats, soybeans, and potatoes) and several weed species including giant foxtail (*Setaria faberi*), barnyardgrass (*Echinochloa crusgalli*, var. *frumentacea*), lambsquarters (*Chenopodium album*) and redroot pigweed (*Amaranthus retroflexus*). A cohort of each crop and weed species in each of the four replicates was artificially infested (Guthrie, 1989) with neonate ECB larvae. For detailed methods for these field experiments see Losey et al. (2001).

All infested plants of each infested species except oats were destructively sampled for ECB overwintering larvae at the end of the growing season. One hundred oat plants were randomly chosen along each of the four infested rows for sampling. All above

ground parts of the plants were dissected and the number of ECB larvae and additional ECB tunnels (without ECB) recorded. For each crop and weed species, stem diameters from a separate sample of 25 uninfested plants were measured with calipers and recorded. For barnyardgrass, the only noncorn host with a substantial density of ECB tunnels, the impact of stem diameter on survival was measured directly, by comparing the stem diameters of 50 randomly chosen uninfested stems, to stem diameters of plants with larvae or tunnels. Data for all plants combined was analyzed by regressing larval survival (the number of larvae and tunnels per plant as a proportion of the original infestation rate) on stem diameter (REG procedure; SAS Institute, Inc., 1990).

Survival and escape from artificial stems. To assess the potential effect of stem diameter on larval survival and escape, larvae were reared in sections of plastic tubing that varied in diameter but contained the same volume of diet. The range of the six tubing diameters used bracketed the range of plant stem diameters that ECB larvae are likely to encounter in the field (Table 1). The effect of tube size on survival without the possibility of escape was assessed in Nalgene™ 890 fluoropolymer (FEP) tubing with a 'hardness' rating of approximately 99. No larvae escaped from this 'hard' tubing. The potential for escape was measured in clear plastic Nalgene™ 180 tubing with a durometer hardness rating of 55 (Shore A). A substantial proportion of the larvae tested chewed out of these 'softer' tubes and escaped.

Each of the six hard tube treatments (diameters 2.4, 3.2, 6.4, 12.7, 15.8, and 25.4 mm) measuring survival was replicated 12 times. All of these hard tubes had a wall thickness of 79 mm. The five soft tube treatments (excluding 15.8 mm) measuring escape was also replicated 12 times in this tube wall thickness. To determine if stem thickness had an effect on escape which might confound interpretation of stem diameter, 12 replications of soft tube treatments (diameters 2.4, 3.2, and 6.4 mm) with thinner walls (1.6 mm) were added. The effect of tube wall thickness did not have a significant effect on escape rate ($P>0.05$) so both thicknesses for each diameter were combined for all subsequent analyses. Thus, for the soft tube treatments measuring escape there were 24 replications of diameters 2.4, 3.2, and 6.4 mm and 12 replications of the 25.4 diameter tubes.

Tubing was cut with a tubing cutter and a cork stopper sealed one end. The appropriate length of each diameter of tubing was calculated using the formula: $3.77\ cm^3 = \pi((d/2)^2)(l)$ where d is the internal diameter of the tubing, l is the calculated length of diet in the tube and $3.77\ cm^3$ is the standard volume of diet to insure complete larval development (Guthrie, 1989, Guthrie et al., 1995 see Table 1 for details of tubing sizes). Tubes were filled with freshly prepared artificial diet three days prior to the addition of ECB larvae and stored under aluminum foil at room temperature. Diet was either injected into the tubes with a disposable syringe (small diameter tubes) or poured in with a small beaker (larger diameter tubes). The largest diameter tubes were filled with 3× the standard diet volume to prevent dehydration of the diet.

All tubes were infested with a single neonate ECB larva on 15 August 1999. The top of the diet in each tube was scored with a scalpel and the first instar applied with a small paintbrush. Each tube was checked under a microscope to confirm that the larvae had entered the diet. Tubes were plugged with a tight wad of nonabsorbent cotton. A small length of air space was left open in each tube between the surface of the diet and cotton. Larvae in tubes were reared in a growth chamber at 27 °C, 75–85% r.h., 16 h of light per day. Long, small diameter tubes were suspended from a shelf bottom with a twist tie; large diameter tubes stood upright. Larvae in tubes were monitored under a microscope every few days and scored as either missing, dead, or survived (to normal pupation) until the completion of the experiment.

Survival and escape (chewing an exit hole and leaving the tube) of ECB larvae were recorded. The effect of diameter on escape (in soft tubes) and survival (in hard tubes) was analyzed with logistic regression and maximum likelihood estimation (CATMOD procedures; SAS Institute, 1990).

Starvation experiments. The length of time that first through fifth instar larvae could survive in the absence of food was assessed in both moist and dry conditions. All larvae were derived from a single batch of ECB eggs obtained from the Department of Plant Breeding colony. First instar larvae received no food and were placed in experimental treatments as they hatched from eggs. Other larvae were reared on artificial diet cubes in plastic boxes following standard rearing procedures (Chareinsuk, 1983). A degree day model (Dittrick & Chiang, 1981) was used to determine when larvae had reached each subsequent instar (second through fifth). Each of the ten treatment combinations (five instars ×two moisture regimes) was

Table 1. Index of diameters of plants, tubes, and ECB larvae and tube lengths

Object	Diameter	Diameter±SEM (mm)[a]	Tube length (cm)
Tube		2.4	85.7[b]
Foxtail		2.8 ± 0.1 a	–
ECB Larva[c]		3.2	–
Tube		3.2	48.0
Oats		3.8 ± 0.1 ab	–
Barnyard grass		3.8 ± 0.2 ab	–
Pigweed		4.2 ± 0.2 b	–
Lambsquarter		4.5 ± 0.3 b	–
Soybean		5.6 ± 0.1 c	–
Tube		6.4	11.9
Potato		12.2 ± 0.3 d	–
Tube		12.7	3.0
Tube		15.8	1.9
Corn		18.5 ± 0.4 e	–
Tube		25.4	2.2[d]

[a]Based on measurement of 25 uninfested plants of each crop and weed species. Actual diameters depicted.
[b]Calculated from the formula $3.77 = 3.14(r^2)$ (l): see methods section for details.
[c]Based on the head capsule width of diapausing 5th instar ECB larvae.
[d]Diet volume increased to 3× standard volume to compensate for diet desiccation.

replicated 20 times with the exception of first instars (17 replications for both moisture regimes).

Larvae were placed individually in 50 × 9 mm FALCON™ disposable Petri dishes with tight-fitting lids. The 'dry' treatment had a dry, Whatman #1, 50 mm® filter paper circle in the bottom of each Petri dish. To provide a source of local humidity in the 'wet' treatment, the filter paper in each Petri dish was lightly misted with distilled water as needed to maintain moisture throughout the duration of the experiment. Petri dishes were placed randomly on a tray in a growth chamber at 20 °C, 40% r.h. with a L14:D10 regime.

Survival data was collected for each day and scored as either live or dead. Mean survival time (days) for each larva was calculated as the average of the last day recorded as alive plus the first day recorded as dead. The experiment on fifth instars was terminated after 29 days as the remaining larvae had entered diapause and were scored as survived.

The effects of larval instar, moisture, and their interaction were analyzed using an analysis of variance (GLM procedure; SAS Institute, Inc., 1990). Data were log-transformed prior to analysis to ensure heteroscedasticity. Differences between means were tested for significance with Bonferroni t-tests.

Larval behavior experiments. Plants used in this study were two to three week-old seedlings grown individually in soil in 20 cm pots in a greenhouse. For each replicate, three pots each of corn, barnyardgrass, and soybean (nine plants total), were placed alternately in a round plastic tub (diam: 19 cm) already 50% filled with soil. There were two circular rows, an inner row of three plants and an outer row of six plants. The tubs were filled with soil to slightly above the rims of the pots, making a uniform and level surface. Each tub was positioned in the center of a small plastic wading pool (diam: 274 cm) filled 50% with soapy water as a barrier against ECB migration between tubs. There were five replicate tubs in each of five identical blocks (blocked by time).

Experiments were carried out in a greenhouse. Ten third-instar ECB larvae were placed on the soil in the center of each tub at mid-morning. Twenty-four h later, individual plants, the soil surface for all tubs, and the water barriers were inspected for larvae and their location was recorded.

The effect of plant species on the proportion of ECB larvae recovered was analyzed as a one-way ANOVA (GLM procedure; SAS Institute, Inc., 1990). The proportion of larvae recovered was compared with the random expectation of 33% with a one-tailed t-test.

Results

Survival on plants. Among infested plants, stem diameter was a significant predictor of ECB survival ($P = 0.0001$) and it explained a significant proportion of the variance in survival ($R^2 = 0.5486$; Figure 1). Stem diameters were dependent on the type of plant ($P \leq 0.001$). Corn, potato, and soybean had different stem diameters, while oat and weed species had more similar stem diameters (Bonferroni t-tests; Table 1).

Stem diameter had a significant effect on infestation rate of barnyardgrass ($P \leq 0.034$). Stem diameters of infested plants were 3.5 ± 0.23 mm ($n = 10$; mean±SE) and significantly larger than stem diameters of control plants ($n = 50$; 2.5 ± 0.2 mm; mean±SE).

Survival and escape from artificial stems. Larval survival in tubes was significantly related to diameter ($n = 144$; $P \leq 0.0001$; $df = 1$; Figure 2) with more larvae surviving in the larger tubes. The proportion of larvae escaping from tubes with lower hardness rating was significantly related to tubing diameter ($n = 84$; $P \leq 0.0001$; $df = 1$; Figure 3) with fewer larvae escaping from the larger tubes.

Starvation experiments. Larval instar had a significant effect on survival ($n = 100$; $P = 0.0001$; $df = 4$). Larger, older larvae survived significantly longer than younger, smaller larvae (Figure 4). Larvae survived longer in moist than in dry conditions ($P = 0.001$; $df = 1$). The effect of moisture on larval survival varied across larval stages ($P = 0.0199$; $df = 4$).

Larval behavior experiments. A significantly higher proportion of larvae were recovered on corn (0.53 ± 0.09) than on barnyard grass (0.23 ± 0.06; $P = 0.035$; $df = 1$) or soybean (0.24 ± 0.08; $P = 0.051$; $df = 1$; Figure 5). The proportion of larvae found on corn was significantly higher than the 33% that would be expected if larvae were foraging randomly among three plant species ($t = 2.54$; $P = 0.026$).

Discussion

One of the major factors affecting the fitness of early instar ECB larvae is their ability to successfully tunnel

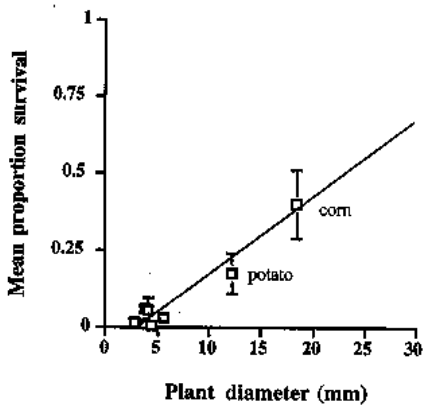

Figure 1. Relationship between stem diameter and ECB larval survival for eight species of plants infested in the field.

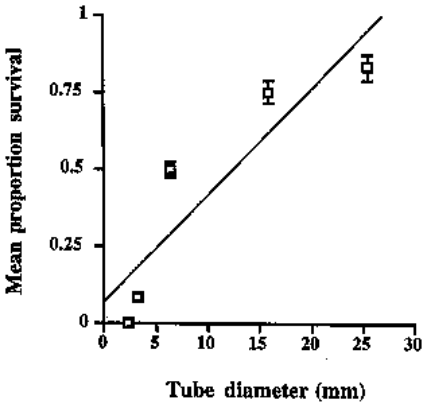

Figure 2. Relationship between stem diameter and ECB larval survival for five sizes of artificial stems (tubes) in the laboratory.

into the stalk of a plant where they can be protected. Our results demonstrate that successful tunnel establishment is correlated with stem diameter both among and within plant species. Across all plants infested with neonate larvae, establishment was highest on corn, which had the largest average stem diameter. Establishment was an order of magnitude higher than on many of the smaller plants. Clearly differences in larval establishment rate among plant species will be affected by many factors other than stem diameter including stem toughness (Beck, 1987). However, the correlation between establishment and stem diameter within a plant species (barnyardgrass) where potentially confounding factors are minimized indicates that stem diameter is useful in predicting establishment.

It appears that ECB larvae may be choosing to colonize plant stems based partly on their perception that the stem is large enough to support complete development. Our results suggest that using stem diameter as one criterion in host plant selection would be an adaptive strategy. We found a strong positive relationship

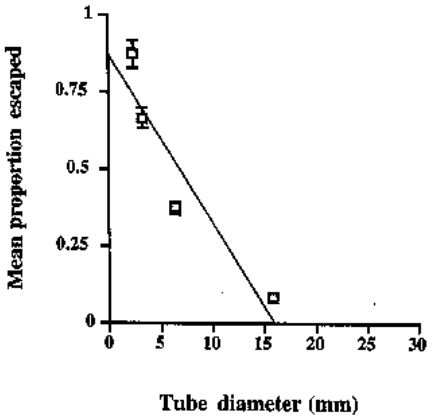

Figure 3. Relationship between stem diameter and the proportion of ECB larvae that escape from four sizes of artificial stems (tubes) in the laboratory.

between diameter and larval survival in experiments designed to isolate diameter from other factors (Figure 2). This indicates a substantial correlation between the preferences of adult and neonate ECB and the performance of subsequent life stages.

It also appears that the propensity of ECB larvae to escape from a plant is correlated with stem diameter. In the field, many entry and exit holes were observed on small-stemmed plants and very few dead ECB larvae were recovered from infested plants (Losey et al., 2001). Our results demonstrate a significant inverse relationship between stem size and the proportion of larvae that escape (Figure 4). As would be expected, ECB larvae show a higher propensity to escape from those tubes that offer a lower probability of survival.

ECB larvae that abandon hosts that do not support complete development face two major challenges, they must manage to survive unprotected outside their original host and they must successfully locate and establish themselves in a host that will accommodate the remainder of development. Several habitat-specific factors will affect the probability of locating a suitable new host, including the proximity of suitable hosts to nursery hosts and biotic (e.g., predation) and abiotic risks (e.g., jagged surfaces) on the substrate between hosts. These factors will interact with larval-specific factors such as the length of time a larvae can survive without food and their ability to perceive suitable hosts from the substrate level. Our results indicate that ECB larval survival in an artificial environment without access to a plant is positively correlated with larval age and that, in general, larvae can survive for a very long time in the absence of food. Even first instar larvae can survive for several days and late instar larvae can

survive for several weeks. Thus, it would appear that once larvae leave a nursery plant they have ample time to locate a suitable host. Clearly late instar larvae have a higher probability of locating a suitable host due to their ability to survive longer without food.

The length of time that an ECB larva must be able to survive in order to successfully colonize a new host will depend largely on its ability to perceive suitable hosts from the substrate level. Even for an herbivore with a fairly wide host range such as ECB the ability to discriminate between suitable and unsuitable hosts will greatly enhance larval foraging efficiency. In our behavioral assays, ECB larvae were found on a suitable host (corn) in significantly higher numbers than would have been expected if they were foraging randomly (Figure 5). These results imply that after ECB larvae abandon their nursery hosts the proportion that colonize corn may be higher than would be expected from a purely random model of dispersal.

Our results have several potentially important implications for the management of resistance to transgenic Bt-corn in ECB populations. One key element in the integrated resistance management (IRM) program for ECB and Bt corn is the presence of a high local density of susceptible individuals to mate with any resistant individuals that arise (Ostlie et al., 1997). To ensure an adequate ratio of susceptible to resistant individuals, the U.S. Environmental Protection Agency has mandated that each field planted with Bt corn be within 0.8 km of an area of non Bt corn that is equal to 20% of the total area of the corn planted (http://www.epa.gov/pesticides/biopesticides/other docs/bt_corn_ltr.htm). Given the wide host range of ECB, it seems plausible that plants other than corn could make some contribution to the pool of susceptibles. However, other studies have established that the proportion of ECB arising from hosts other than corn is so small that they constitute an insignificant portion of the susceptible ECB population (Losey et al., 2001). Based on our results, it appears that the probability of ECB boring into and completing development in plants with stem diameters significantly smaller than corn is low. In other studies pupation of ECB larvae has been observed in the field on plants as small as wheat (Willson, 1980) but that report also notes that larval exit holes were frequently observed. This apparent inability of a substantial proportion of ECB larvae to develop completely on plants smaller than corn greatly limits the set of plant species that are likely to make a substantial contribution to the pool of susceptible ECB adults.

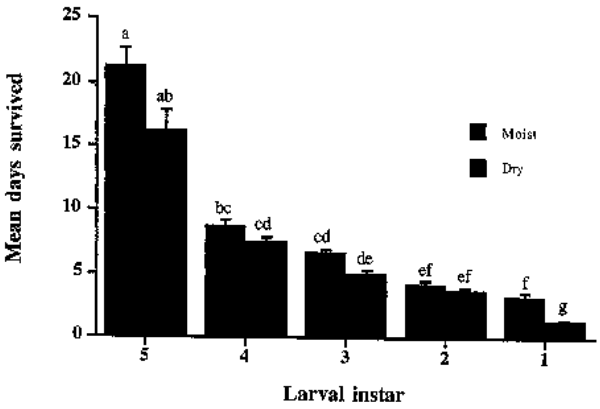

Figure 4. Mean (±SEM) survival of five instars of ECB larvae held without food in moist or dry conditions. Means labeled with the same letter are not significantly different at P = 0.05.

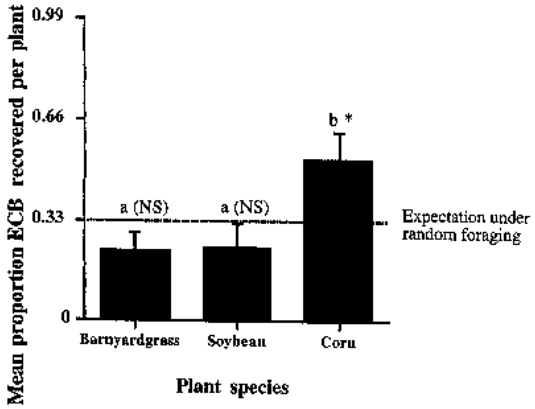

Figure 5. Mean (±SEM) proportion of ECB larvae recovered from three species of plants in a foraging assay. Means labeled with the same letter represent plants that are not significantly different at P = 0.05. Each plant mean is labeled with * for significant or NS for not significantly different from a proportion of 0.33.

Furthermore, our results suggest that smaller plants serving as nursery hosts for ECB could actually speed the development of resistance. ECB on nursery hosts are likely to escape from those hosts, and once they do they can survive for long periods off of plants and forage for corn effectively. Nursery plants may allow partially resistant individuals to develop to later instars that can better tolerate Bt corn (Huang et al., 1999). One of the goals of the IRM program for Bt corn is to eliminate these partially resistant individuals by utilizing plants that express the Bt toxin at a 'high dose'. By facilitating the early survival of these partially resistant individuals, nursery hosts may facilitate the development of full resistance. Based on our results it appears that the role of nursery plants may need to be factored more explicitly into IRM programs for Bt corn.

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